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Richardus F. Kaswadji

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**The evaluation and comparison of aquatic methodology for
measurement of bacterial population dynamics and grazing
activity in diverse aquatic ecosystems**

Kaswadji, Richardus F., Ph.D.

The Louisiana State University and Agricultural and Mechanical Col., 1991

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**300 N. Zeeb Rd.
Ann Arbor, MI 48106**

**THE EVALUATION AND COMPARISON OF AQUATIC METHODOLOGY FOR
MEASUREMENT OF BACTERIAL POPULATION DYNAMICS AND GRAZING
ACTIVITY IN DIVERSE AQUATIC ECOSYSTEMS**

A Dissertation

**Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy**

in

The Department of Oceanography and Coastal Sciences

by

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ABSTRACT

Observations and experiments were conducted to evaluate methodologies for measuring aquatic bacterial population dynamics and grazing activity in ecologically dissimilar ecosystems. Samples were taken from Auke Bay, AK, the Mississippi River at Plaquemine, LA, the Terrebonne Bay estuary, LA, and the Mississippi River plume. Four methods were compared for measuring bacterioplankton growth and grazing mortality rates: dilution, filtration, antibiotic, and the Servais et al. (1985) procedures. The dilution method appeared to be the best among those compared.

Among all sites, bacterial density ranged from 0.02 to 10^6 ml^{-1} , and bacterial growth and grazing mortality rates, estimated using the dilution method, ranged from -0.016 to 0.11 h^{-1} and 0.012 h^{-1} to 0.134 h^{-1} , respectively. The number of bacteria grown and grazed in all study locations were nearly in balance, implying that grazers can increase in magnitude equivalent to bacterial growth.

Grazing is the major factor controlling bacterial density in all study areas, although at particular sites temperature and food availability also have an important role. Seasonal changes in bacterial abundance and growth rate were not a function of temperature in Auke Bay and Terrebonne Bay estuary, but were in the Mississippi River. Phytoplankton are important as a source of carbon for bacteria in Auke Bay, but perhaps not in the Lower Mississippi River.

There were 8% per hour turnover of bacterial biomass (ranged of 1 to 20% per hour) among all sites. In Terrebonne Bay, each flagellate grazed between 13 to 86 (average 23) bacteria per hour. There were virtually no flagellates present when bacterioplankton densities were less than $0.5 \times 10^6 \text{ ml}^{-1}$, suggesting that there are at least two qualitatively different bacterial population dynamics in aquatic environments: one with, and one without significant grazing by flagellates. When there is an absence of grazing, bacteria are a

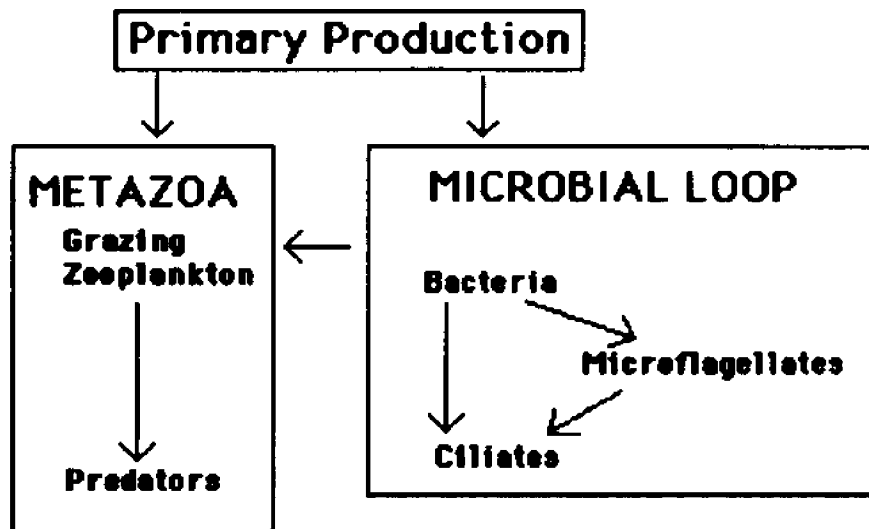
"sink". In the presence of grazing, bacteria may be a "sink" or a "source" ("link") of organic matter for the higher trophic levels. The value of comparisons in widely dissimilar aquatic ecosystems has been demonstrated.

CHAPTER 1

INTRODUCTION

More than 15 years ago, Pomeroy (1974) substantially revised the paradigm of the roles of bacteria in aquatic food webs. Before that, in the classical or traditional paradigm of the planktonic food web, the roles of bacteria were largely minimized or even ignored. The earlier view held that energy and matter were transferred sequentially to higher trophic levels through phytoplankton which were captured by zooplankton, which in turn were consumed by small fish, the latter eaten by even larger fish. About 10 to 50% (Larsson and Hagstrom, 1982) of the carbon fixed in primary production was lost into the water as DOC (dissolved organic carbon). The new paradigm suggested that bacterioplankton played a significant role in this process by taking up the DOC as an energy source for their production. Coupled with grazing by microflagellates (and ciliates), bacterioplankton returned some of the lost DOC to the main food chain. To simplify the complicated processes of these transfers of carbon, Azam et al (1983) introduced the term "microbial loop". Thus, the field of science called "microbial ecology", the study of the interrelationships among microorganisms and their environments was established. The following diagram, adapted from Pomeroy and Wiebe (1988), shows the position of the microbial loop in the planktonic food web.

Methods to determine bacterial numbers and biomass have developed rapidly within the last decade, resulting in diverse approaches for measuring bacterioplankton production and mortality. Understanding of the role of bacteria in aquatic ecosystems was improved with development of these methodologies and their application in marine and freshwater ecosystems in both cold and warm environments. However, the dissimilar results from a variety of methods available sometimes added confusion in deciding which method to choose and how to interpret the results.



The paucity of simultaneous measurements of bacterial growth and grazing rates also has inhibited a comprehensive synthesis comparing bacterioplankton population dynamics in various dissimilar environments.

To address this problem, experiments were conducted in several different environments, including Auke Bay, Alaska, the Mississippi River at Plaquemine, Louisiana, the Terrebonne Bay estuary, Louisiana, and the Mississippi River plume.

Auke Bay is a semi-enclosed embayment in southeastern Alaska (58° 22' N; 134° 40' W), approximately 20 km from Juneau. It is a well-ventilated estuary and relatively well-flushed with adjacent waterways (Coyle and Shirley, 1990). The average depth is 40 m in the bay and 8 m at the sampling site. The range of temperature and salinity during the study were 3 to 10° C and 14 to 34 ppt, respectively, and the chlorophyll concentration ranges from 0.23 to 15 µg/l. Auke Creek, Auke Nu Creek, Wadleigh Creek and several unnamed streams, fed by rain and melt water from the snow pack, drain into Auke Bay. Lemon Creek and the Mendenhall River, fed by melt water from the Mendenhall Glacier, drain into Fritz Cove, adjacent to Auke Bay (Coyle and Shirley, 1990). Auke Bay appears to be a phytoplankton dominated system, highly productive, and dominated by either

Thalassiosira aestivalis, Skeletonema costatum, or Chaetoceros compressus during the spring phytoplankton bloom (Ziemann, 1990). Auke Bay was the site of the APPRISE (the Association of Primary Production and Recruitment In Subarctic Ecosystems) study that was held between 1985 and 1989 (Ziemann, 1990). The laboratory in which this present study was conducted is at the edge of the bay.

The Mississippi River at Plaquemine is a terminus of the largest river in the United States, with man-made flood protection levees on each side. It is a turbid water (secchi disc reading < 0.5 m) with annual temperature and chlorophyll ranges of about 2 to 30° C and 0.5 to 5 µg/l, respectively. This ecosystem appears to be detritus dominated, with most detritus originating from upstream. There are no macrophytes of consequence on the shoreline. The water samples for the present study were taken at the middle of the river from a ferry.

Terrebonne Bay estuary is a shallow estuary (average depth 5 m) with barrier islands separating it from the Gulf of Mexico. The temperature, salinity, and chlorophyll ranges at this system are about 15 to 32° C, 2 to 30 ppt, and 2 to 20 µg/l, respectively. Terrebonne Bay appears to be a detritus based system, and the sampling site, near the LUMCON Laboratory was surrounded by vast expanses of Spartina alterniflora, a highly productive salt marsh plant species (Kaswadji et al., 1990).

The offshore samples were taken from the Mississippi River plume and from offshore of Louisiana in the Gulf of Mexico. Twenty five percent of US fisheries landings are from this zone. The sample depth range was 5 to 100 m, with the ranges of temperature, salinity, and chlorophyll from 15 to 32° C, 0 to 36 ppt, and 2 to 50 µg/l, respectively. This site is a hypoxic zone during the summer ($O_2 < 2$ mg/l). Carbon in this area comes from the Mississippi River and from in situ sources.

The contrasting salinity, temperature, carbon sources, and turbidity (see also Table 1.1) represent a variety of environmental conditions in which bacterial communities could adapt, grow, and be grazed upon. A broad range of results obtained from a common

Table 1.1.. The ranges in salinity, temperature, Chl *a*, and depth in each environments studied; dates of sample collection and the number of samples are also given.

Sample Location	n	Range Salinity (ppt)	Range Temperature (°C)	Range Chl <i>a</i> (µg/l)	Range Depth (m)	Dates of Sample Collection
Auke Bay, AK (58° N)	24	14-34	3.0-10.0	0.234 - 15.085	6-13	Apr '90-May '90
Lower Mississippi River, Plaquemine, LA	18	0-0	2.0-30.0	0.50 - 5.0	NA	Oct '89-Oct '90
Terrebonne Bay, Cocodrie, LA	11	9-15	17.0-32.0	2.0 - 20.0	1-3	Aug '89-Dec '90
Mississippi River Plume	7	0-35	21.0-22.0	2.0 - 50.0	5-100	Sep '89

NA = not available

methodological approach was thus sought to discern and observe general population behavior. The objectives of the experiments were to answer the following questions that arose during the literature review:

1. Which is the best method among the methods available for measuring bacterial growth and grazing mortality rates?
2. What is the quantitative relationship between bacterial growth and mortality?
3. What are the major factors influencing bacterial density?
4. How closely are grazers and bacteria coupled?
5. Are bacteria a "sink" or a source of food for the higher trophic levels?

The four composite investigations of this dissertation are the results of the experiments mentioned above. Chapter 2 compares four different methods (dilution, filtration, antibiotic, and Servais et al.) to calculate bacterial growth and grazing mortality. Except for the Servais et al. (1985) method, the other methods were based on temporal changes of bacterioplankton density over 24 hours. The study was intended to find out which of the methods examined was the best for estimation of bacterial production and mortality. For this purpose, results of measurements from cold marine (Auke Bay, AK), warm marine (Gulf of Mexico), estuarine (Terrebonne Bay estuary, LA), and riverine (Mississippi River, LA) environments were compared.

Chapter 3 is a report of results of a study on microbial growth and grazing mortality during a spring phytoplankton bloom in Auke Bay, southeastern Alaska. Since bacteria utilize carbon released by phytoplankton as an energy source, a high density of phytoplankton during the bloom would hypothetically affect the bacterial dynamics. It appeared that the phytoplankton bloom in this study occurred earlier than expected, making it necessary to examine the dynamics at the end rather than during the bloom.

Chapter 4 presents results of a study on bacterial growth and grazing mortality in the Mississippi River, near Baton Rouge, LA. The bacterial dynamics were monitored

over one full year, from October 1989 to October 1990, to investigate the physical and biological factors controlling bacterial density.

Chapter 5, reports on experiments dealing with grazing of microflagellates on bacteria. Microflagellate density was calculated along with bacterial temporal density changes to determine if grazing activity was related to food (bacteria) availability.

Chapters 2 and 3 have been prepared for publication. Chapter 3 has been presented as a poster in the 91st General Meeting of the American Society for Microbiology in Dallas, TX (May 5 - 9, 1991). Chapters 4 and 5 will be submitted to appropriate journals for publication.

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CHAPTER 2

A COMPARISON OF THE DILUTION, FILTRATION, ANTIBIOTIC, AND ISOTOPIC TURNOVER METHODS USED TO MEASURE BACTERIOPLANKTON DYNAMICS

Introduction

Rapid progress in method development has resulted in diverse approaches to measure bacterioplankton growth, grazing mortality, and net growth rates. These methods now include: (1) estimation of frequency of dividing cells (FDC; Davis and Sieburth, 1984; Hagstrom et al., 1979); (2) physical separation of bacterial prey from their predators using 1.0 μm filters (Fuhrman and Azam, 1982); (3) a series of dilutions to reduce prey and predator encounters (dilution method, Landry and Hassett, 1982; Ducklow and Hill, 1985); (4) inhibition of prey growth, but not predator activity, through non-lethal antibiotics (Bjornsen, 1988; Taylor and Pace, 1987; Sanders and Porter, 1986); (5) isotopic tracers such as thymidine and leucine (Riemann et al., 1990; Pomeroy et al., 1990; Kirchman and Hoch, 1988; Chin-Leo and Kirchman, 1988); (6) genetic markers or "mini-cells" (Wikner et al., 1986); (7) disappearance of incorporated tritiated thymidine attributable to mortality losses (Servais et al., 1985); and, (8) uptake or digestion of stained real or artificial bacteria (Sherr and Sherr, 1987). The widely-used TTI (tritiated thymidine incorporation) method of measuring growth rates is often field-calibrated using one of these other methods. Azam and Fuhrman (1984), among others, have argued for comparative evaluations of these methods. Newell and Fallon (1982) used the FDC, TTI, and 3- μm filtration methods to measure bacterial productivity in the water column and sediments in Georgia. Fallon et al. (1983) compared the results of bacterial production in marine sediments using the FDC and TTI approaches. McManus and Fuhrman (1988) reviewed grazing mortality measurements using the dilution, filtration, inhibition, and Servais et al. (1985) methods.

Reported here are the results of several procedures used to follow bacterioplankton dynamics from samples collected in arctic and temperate environments. Four different methods were used to measure bacterial growth rates and bacterial grazing mortality rates (dilution, filtration, antibiotic, and Servais et al.(1985) methods). The validity of various assumptions necessary to accept the results from these methods was investigated.

Materials and Methods

Water samples were taken from Auke Bay, AK, the Mississippi River at Plaquemine, LA, the Terrebonne Bay estuary, LA, and the Mississippi River plume. Samples from Auke Bay are from the National Marine Fisheries Services Auke Bay Lab dock. Mississippi River water was sampled in the middle of the river from the Plaquemine Ferry. Water from Terrebonne Bay estuary was sampled at Bayou Price near the Louisiana Universities Marine Consortium (LUMCON) pump house, Cocodrie. Other samples were taken from the R/V Pelican off the mouth of the Mississippi River.

Surface water was collected using either a bucket or Niskin bottle (Hydro Products Model No. XRB-135), its temperature and salinity were measured immediately, and then was brought to the laboratory for experiments. The four different methods used in these experiments were dilution, filtration, antibiotic, and Servais et al.(1985).

Dilution method (Landry and Hassett, 1982).

Water samples were diluted to a final volume of 250 ml with diluent to make up a 100, 50, 20, 10, 5, 2, and 1% solution of the original water sample. The diluent was prepared by filtering the original water sample through GF/C and 0.2 μm Nuclepore filters under <100 mm Hg pressure. The various fractions were then incubated in the dark at the in situ temperature and 2 ml subsamples were taken at 0, 5, 10, 15, 20, and 24 hours. The bacteria cell numbers (N_t) in each subsample were enumerated, and the $\ln(N_t)$ regressed

against time (t). The slope of the regression line was assumed to be the apparent bacterial growth rate. This growth rate is actually the result of the real growth rate minus the grazing and non-grazing mortality rates. These apparent growth rates were designated k_n , where n is the 100, 50, 20, 10, 5, 2, and 1% fraction of original water sample. Thus, k_{100} was the apparent growth rate of bacteria when the density of grazers was 100% of that in the original water sample.

The purpose of diluting the sample was to provide a series of conditions in which there were less encounters between bacteria and grazers. K_0 was the growth rate of bacteria when no grazers were present (instantaneous growth rate, μ) and was estimated as the intercept of the regression line of k_n and the dilution factor. The slope of the regression line was the bacterial grazing mortality rate. Thus, the dilution method can be used to estimate both bacterial growth and grazing mortality rates.

There are three assumptions that must be met to accept results from the dilution method: (1) the change in bacterial number over time follows an exponential model (e.g., Ducklow and Hill, 1985); (2) the reduction in grazing mortality is directly proportional to the dilution (Landry and Hassett, 1982); and, (3) bacterial growth rates are not affected by the dilution treatment (Ferguson et al., 1984).

Filtration method (Ducklow and Hill, 1985; Coffin and Sharp, 1987).

Water was filtered through GF/C and 1 μm Nuclepore filters for samples from the Mississippi River, and through 1 μm filter for those from LUMCON and Auke Bay. Two hundred and fifty milliliters of the filtered water were incubated in the dark at the in situ temperature. Two milliliter subsamples were drawn at 0, 5, 10, 15, 20, and 24 hours. The bacterial cell numbers of each subsample were then enumerated and $\ln(N_t)$ were regressed against time. Since most, if not all, free bacteria are less than 1 μm and most bacterioplankton protozoan grazers are greater than 1 μm , filtering the water sample through a 1 μm filter should separate free bacteria from their predators. The increase in the

bacterial cell number in the filtered sample was considered to represent bacterial growth. The slope of the regression line was the bacterial growth rate, or $k_{1\mu m}$. When this method is used without benefit of additional information, it results in only an estimate of bacterial growth rate. However, bacterial grazing mortality was estimated by subtracting k_{100} , which was determined from the dilution method, from $k_{1\mu m}$. Results from the filtration method were accepted when it could be assumed that filtration and removal of grazers did not affect bacterial growth rates (Fuhrman and Azam, 1980).

Antibiotic method (Bjornsen, 1986).

This method uses a combination of antibiotics to inhibit bacterial production (Bjornsen, 1988) with the assumption that grazers are unaffected. A combination of 50 mg per liter each of penicillin and streptomycin was added into a 250 ml water sample. The water sample was then incubated in the dark at the in situ temperature and 2 ml subsamples were drawn at 0, 5, 10, 15, 20, and 24 hours. The bacterial cell number in the subsample was enumerated. The slope of the linear regression of $\ln(N_t)$ vs time was presumed to be the result of grazing losses. For simplification it was defined as k_{PS} , where k is the grazing rate, for an experiment using penicillin (P) and streptomycin (S). This method measured the grazing mortality rate only. However, an estimate of bacterial growth rate is obtained if k_{PS} is subtracted from k_{100} obtained from the dilution method.

Servais et al. method (Servais et al., 1985).

Servais et al. (1985) developed a method to follow the disappearance of [3H]-thymidine from live cells once all of the [3H]-thymidine was gone from solution. The total bacterial mortality rate is presumed equal to the disappearance rate of the isotope from the particulate matter. A volume of 3.8 ml of [3H]-thymidine was added to a 250 ml water sample, and incubated in the dark at the in situ temperature. After 10 hours, 10 ml of subsample was collected and put into a test tube. About 0.15 ml of diluted thymidine was

added to the sample, which was then incubated in the dark for 30 minutes. The incubation was terminated by placing the test tube on ice for one minute, after which 1 ml of cold 50% TCA was added (final concentration of 5%). After five minutes of extraction on ice, the sample was filtered onto 0.2 μm Nuclepore filter blackened with Irgalan Black, and rinsed twice with cold 10% TCA. At this time, thymidine incorporated into the DNA and other macromolecules remain on the filter. The filter was removed from the filtering tower and placed in a scintillation vial, and a 15 ml cocktail (MULTISOL, Isolab) was added. Other subsamples were collected at 20, 30, 40, 50, 80, and 120 hours. The dpm (disintegration per minute) of each subsample was measured and a linear regression of the \ln (dpm) versus time determined. The regression slope was the total mortality rate of the bacterioplankton (grazing and lysis). This method assumes that the bacterial growth and mortality rates remain unchanged during the experiments (up to five days).

Bacteria enumeration.

The enumeration of the bacterial density in all of the above methods was done using the direct count technique with Acridine Orange (AO) (Hobbie et al., 1977). Two milliliters of subsamples were pipetted into 4-ml sample cups, and 0.2 ml of formaldehyde was added immediately into each cup to stop bacterial production and grazing mortality. The preserved subsamples can be stored for several weeks before enumeration (Daley and Hobbie, 1975). Immediately before enumeration, the subsamples were stained with 0.2 ml of 0.1% AO for about three to four minutes, following which they were vacuum pumped at very low pressure (≤ 100 mm Hg) onto a 0.2 μm black-Nuclepore filter, and rinsed twice with distilled water. All liquids used in this procedure (i. e., formaldehyde, acridine orange, and distilled water) were filtered through 0.2 μm filters. The filters were mounted onto glass slides and observed at 1500x. A Nikon Optiphot Epifluorescent microscope equipped with a 100 W halogen lamp and blue filter was used to count the bacteria which fluoresced bright green.

The dilution, filtration, and antibiotic methods estimate bacterial growth and grazing mortality rates based on the changes of Bn (the number of bacteria) over time (24 hours in this case). Thus, in addition to carefulness in preparing the experiments, the accuracy of bacterial enumeration is very important. There are several ways to maximize counting accuracy. Many investigators examine at least 10 microscopic fields or count up to 200 (Hobbie et al., 1977) or 300 bacteria (Hanson et al., 1983; Andersen et al., 1986). Kirchman et al. (1982) put a 0.45 μm Millipore filter beneath the 0.2 μm black Nuclepore filter to facilitate an even distribution of cells, and then counted 10 randomly selected fields with 50 or more cells per microscope field. Several preliminary enumerations of bacteria at various numbers of fields (Fn) per slide were done and the standard deviations (Std) within sample variation calculated (Fig. 2.1). The Std was the highest at $\text{Fn} < 15$, and stabilized at $\text{Fn} \geq 20$. Therefore, 20 fields were counted to determine bacterial density for the remainder of the study. Of the first 26 duplicate counts, the average range was 12% of the mean between the duplicates; thus, it was decided that duplicates were not needed for bacterial enumerations.

Growth rates were calculated on the basis of estimates of changes in bacterial density over 24 hours and assuming logarithmic growth. It was necessary to optimize counting effort by minimizing the number of subsamples taken. Therefore, the relationship of accuracy to sampling effort was determined with the goal of perhaps reducing effort. A total of 13 experiments were conducted in which Bn was determined at 0, 5, 10, 15, 20, and 24 hrs. From these, the apparent growth rates were calculated using sampling rates of 2 (at zero and 24 hr), 3 (at 0, 5 or 20, and 24 hr), 4 (at 0 and 24 hr, and 2 others in between), and 6 (all 6 subsamplings). The apparent growth rates resulting from using 2, 3, and 4 samplings were compared against those using all 6 data (Fig. 2.2). The y-intercepts were 0.005, 0.004, and 0.005 for 2, 3, and 4 samplings, respectively. The slope of the regressions for 2, 3, and 4 samplings were 0.952, 0.978, and 0.968, respectively, and were not significantly different from 1.0 and from each other (t-test).

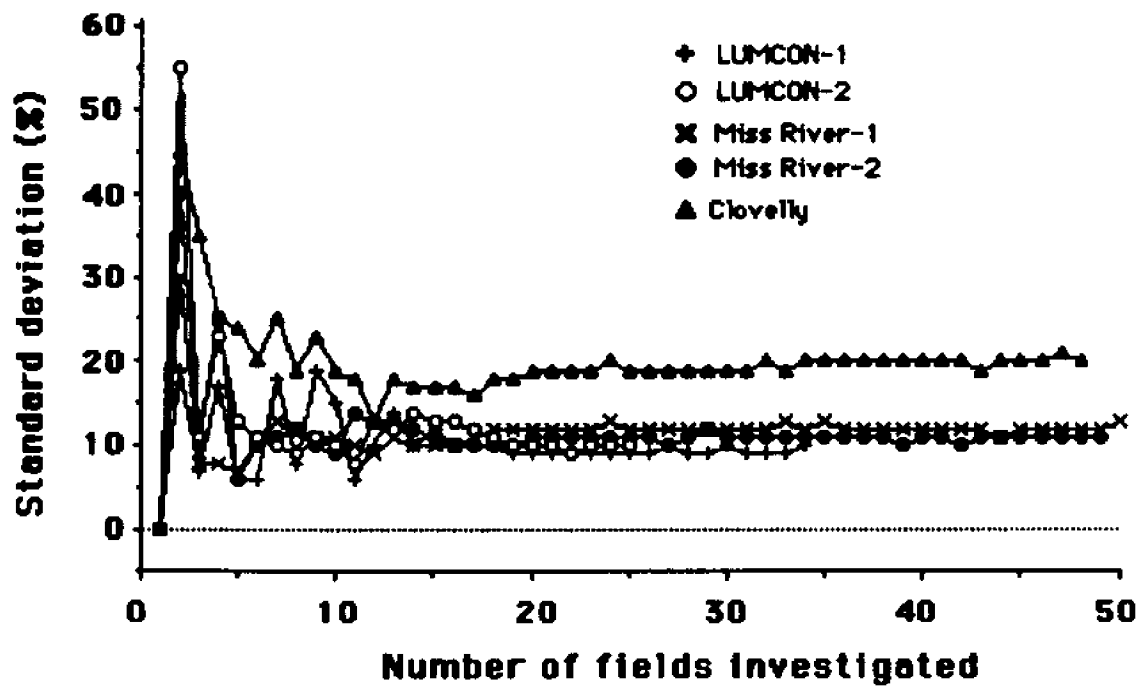
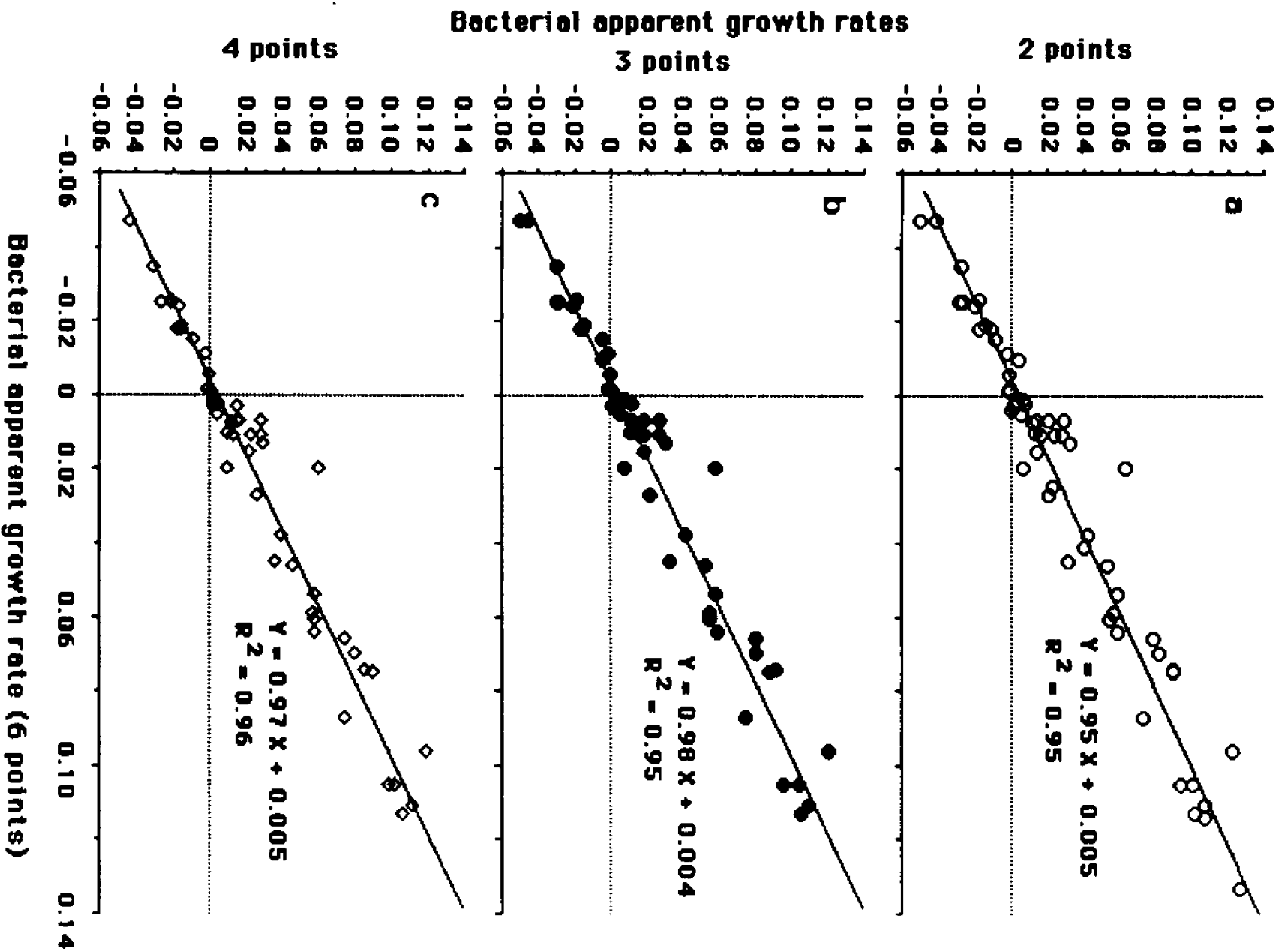


Figure 2.1. The standard deviation (%) of the number of bacteria at different cumulative numbers counted.

Figure 2.2. The apparent growth rates estimated using two (a), three (b), and four (c) points (subsamples) compared to those estimated using six points (subsamples).



$p < 0.05$). Based on these findings, subsamples were taken at 0, 5 or 20, and 24 hr, thereby cutting by 50% the time spent to obtain the same results as those by sampling 6 times.

Results and Discussion

Filtration losses.

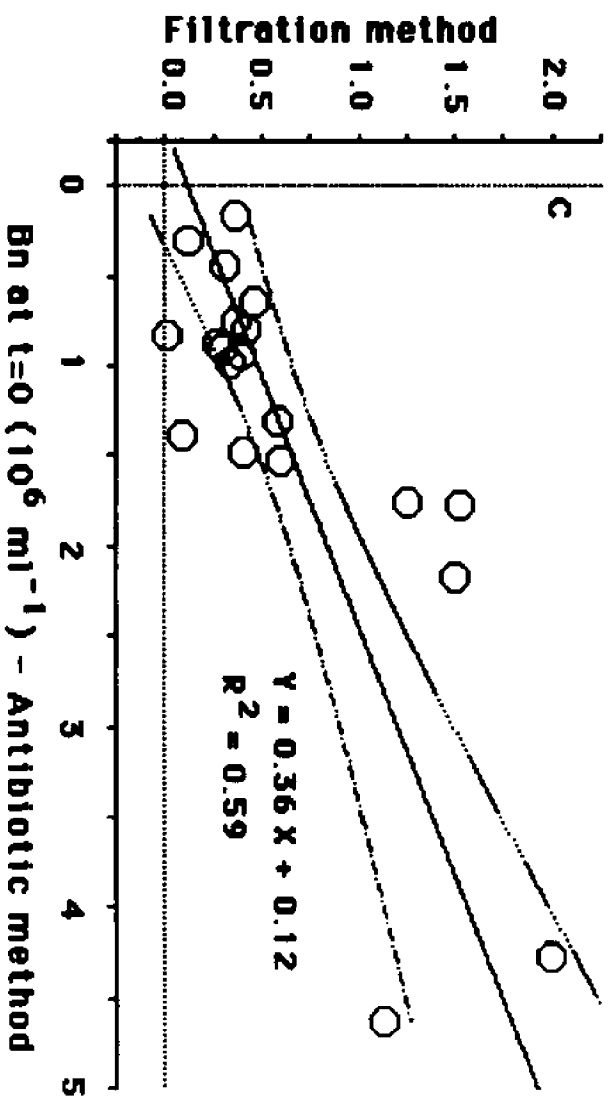
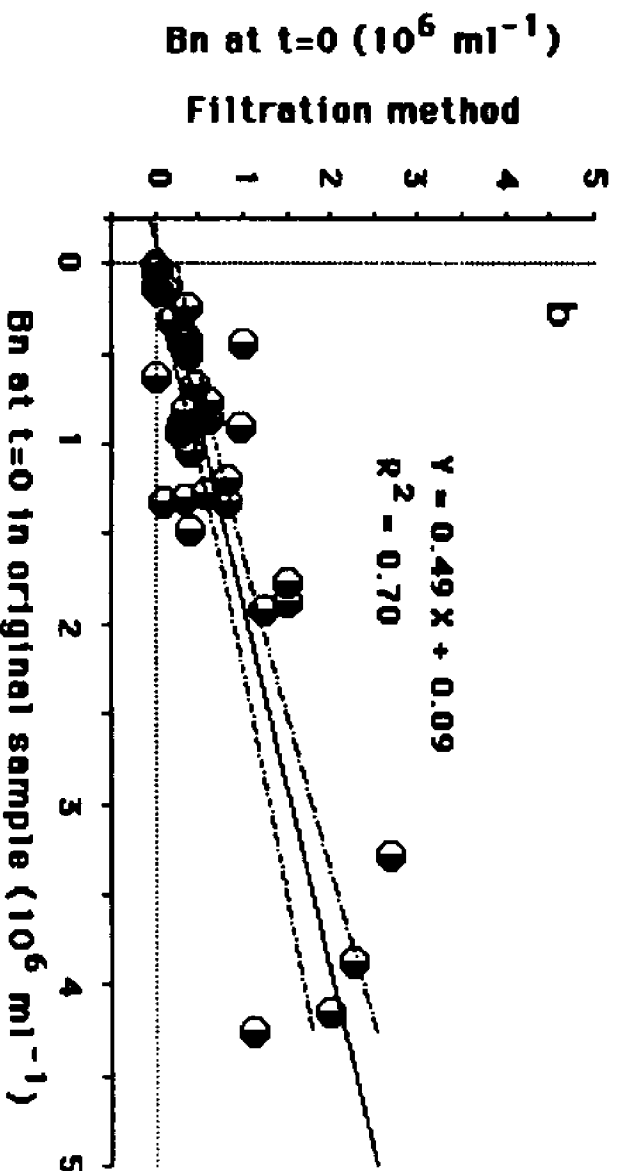
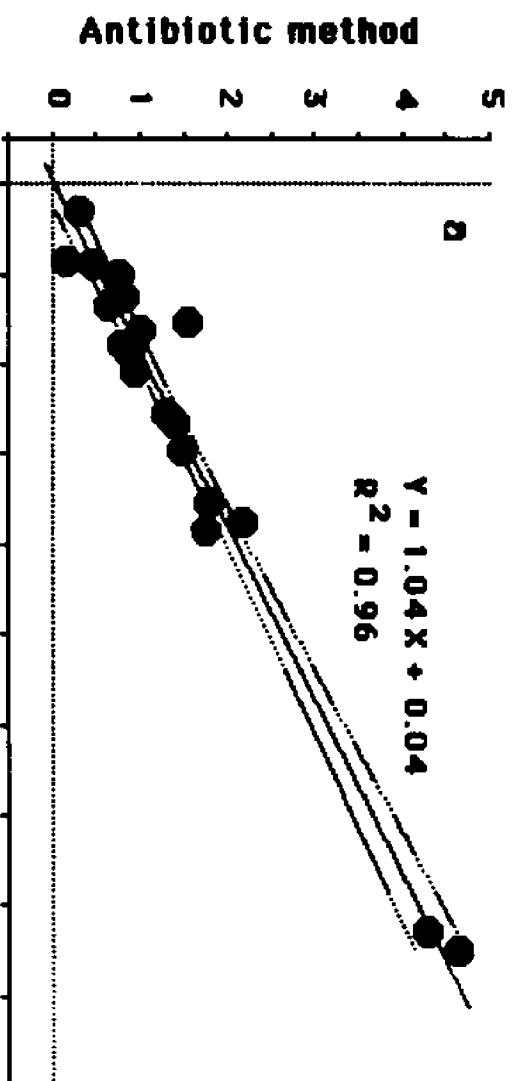
The number of bacteria at the beginning of experiments among the dilution, filtration, and antibiotic methods was compared. B_{OR} (Bn in unaltered sample at $t=0$) is comparable to B_{PS} (Bn in antibiotic experiment at $t=0$) (Fig. 2.3a) as expected, since there were no treatments that could cause any differences for both at the beginning of the experiment. On the other hand, B_{FILT} (Bn in filtration experiment at $t=0$) was almost always lower than both B_{OR} and B_{PS} (Fig. 2.3b, c). Furthermore, F-tests showed that there were highly significant differences between B_{FILT} and both B_{OR} and B_{PS} . The number of bacteria in B_{FILT} was about 50-60% less. The likely explanation for these differences is that the filters trap bacteria.

Dilution method.

One assumption of the dilution method is that bacteria will grow exponentially over time in the absence of grazers. To test the validity of this assumption, the shape of the bacterial growth curve for water passed through 1 μm pore-size filter was examined. If bacterial growth was exponential, then there must be a strong linear regression correlation ($R^2 \geq 0.80$) between the natural log of Bn versus time. Thirty eight out of 39 experiments (97%) had an $R^2 \geq 0.80$ (Fig. 2.4). Therefore, this assumption was accepted as valid.

A second assumption is that a reduction in grazing mortality is directly proportional to the degree of dilution. The R^2 of the linear regression model of the apparent growth rate

Figure 2.3. B_n at $t=0$ for the original sample versus results from the antibiotic (a) and filtration (b) methods, and for results from the filtration versus antibiotic methods (c); the curved lines are the 95% confidence bands for the true mean of Y .



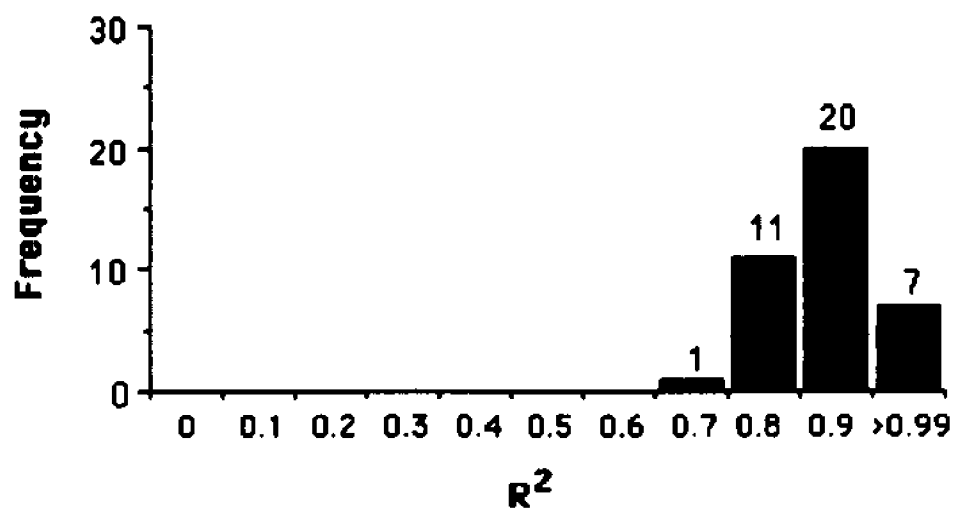


Figure 2.4. The frequency distribution of samples within the observed range of R^2 for a linear regression of $\ln(Bn)$ versus time.

versus dilution factor should be high (≥ 0.80) if bacterial growth is grazer-limited. Ninety-two percent (36 out of 39) of the R^2 values for a linear regression of the bacterial apparent growth rates versus dilution factor were ≥ 0.80 (Fig. 2.5). Therefore, the second assumption was considered valid.

A third assumption is that the dilution treatment does not affect the bacterial growth. The carbon enrichment into the diluent arising from cell lysis during filtration may enhance bacterial growth in diluted samples (Ferguson et al., 1984). The third assumption was accepted on the basis of the work of Tremaine and Mills (1987). Tremaine and Mills found that the impact of the carbon enrichment (16% of the variance) was low compared to the impact of dilution (64% of the variance) on bacterial growth. They concluded that the bacterial growth rates in the diluted water were not significantly different from that of the control.

Filtration method.

In this method it is assumed that the process of filtration itself and the removal of particles larger than $1\text{ }\mu\text{m}$ does not change the bacterial growth rate. These two assumptions were not directly tested in this study. However, Fuhrman and McManus (1984) presented indirect evidence that grazers can pass $0.6\text{ }\mu\text{m}$ pore-size filters. Furthermore, they observed inhibition of bacterial growth after being filtered through $1.0\text{ }\mu\text{m}$ pore-size filters. Thus, the filtration method may underestimate growth and overestimate grazing mortality rates when the mentioned conditions exist. The effect of filtration on growth rates was indirectly estimated by a comparison of the growth rates determined from the dilution and the filtration methods (Fig. 2.6). Since there was no good agreement between the two, it was concluded that the assumption is not valid and that filtration may change growth rates.

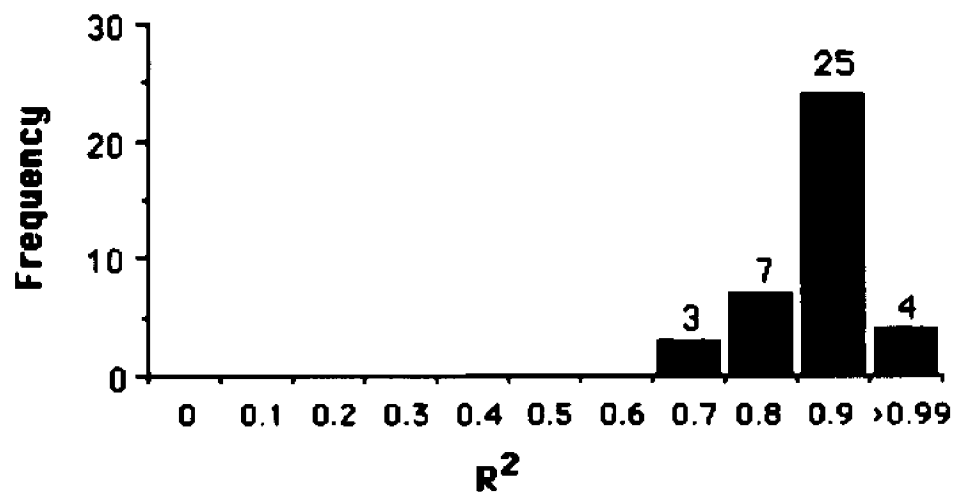


Figure 2.5. The frequency distribution of samples within the observed range of R^2 for a linear regression of the apparent growth rates versus the dilution factor.

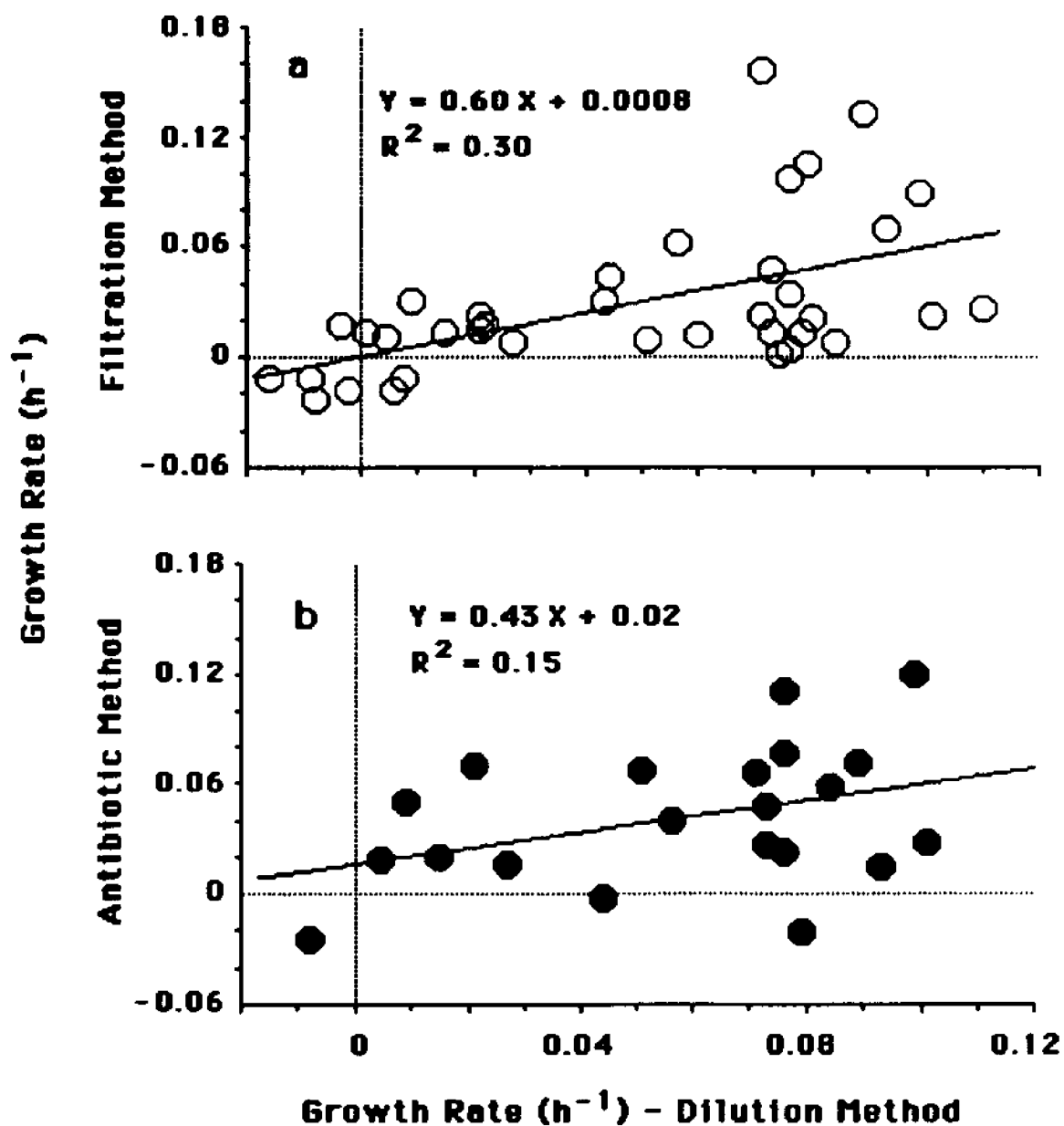


Figure 2.6. The relationship between growth rates estimated using the dilution method versus the filtration and antibiotic methods.

Antibiotic method.

The use of results from this method requires acceptance of the assumption that addition of antibiotics completely inhibits bacterial growth but not grazing losses. A preliminary study was done by adding penicillin, streptomycin, and the combination of the two into unfiltered and 1 μm filtered water. Results are shown in Figure 2.7. The combination of 50 mg/l each of penicillin and streptomycin inhibited bacterial growth the most. Growth was not completely inhibited, but was very low (0.0004 hr^{-1}). Figure 2.7b shows the effect of antibiotics on grazing mortality. Again, the combination of 50 mg/l each of penicillin and streptomycin had a maximum inhibition of bacterial growth, resulting in the highest bacterial grazing mortality rate (0.049 hr^{-1}). Since the antibiotics did not completely inhibit growth, results obtained using this method would provide underestimations of grazing mortality and overestimations of growth rates.

Servais et al. method.

The Servais et al. (1985) method requires the longest incubation time of the four methods discussed. This method assumes that bacteria growth is unaffected over an incubation period of up to five days. This assumption was tested by comparing total mortality rates estimated from the Servais et al. method to the grazing mortality rates estimated from the dilution and filtration method. The results show a poor agreement between methods, with the Servais et al. method yielding relatively low estimates of mortality (Fig. 2.8).

Comparison of methods.

The results of growth and mortality rates using each method are shown in Tables 2.1 and 2.2 with the main points summarized in Table 2.3 arranged by site sampled. Some 'negative' bacterial growth rates were obtained using the dilution and filtration methods, all of which were from low temperatures. Low growth rates at low temperatures (Pomeroy

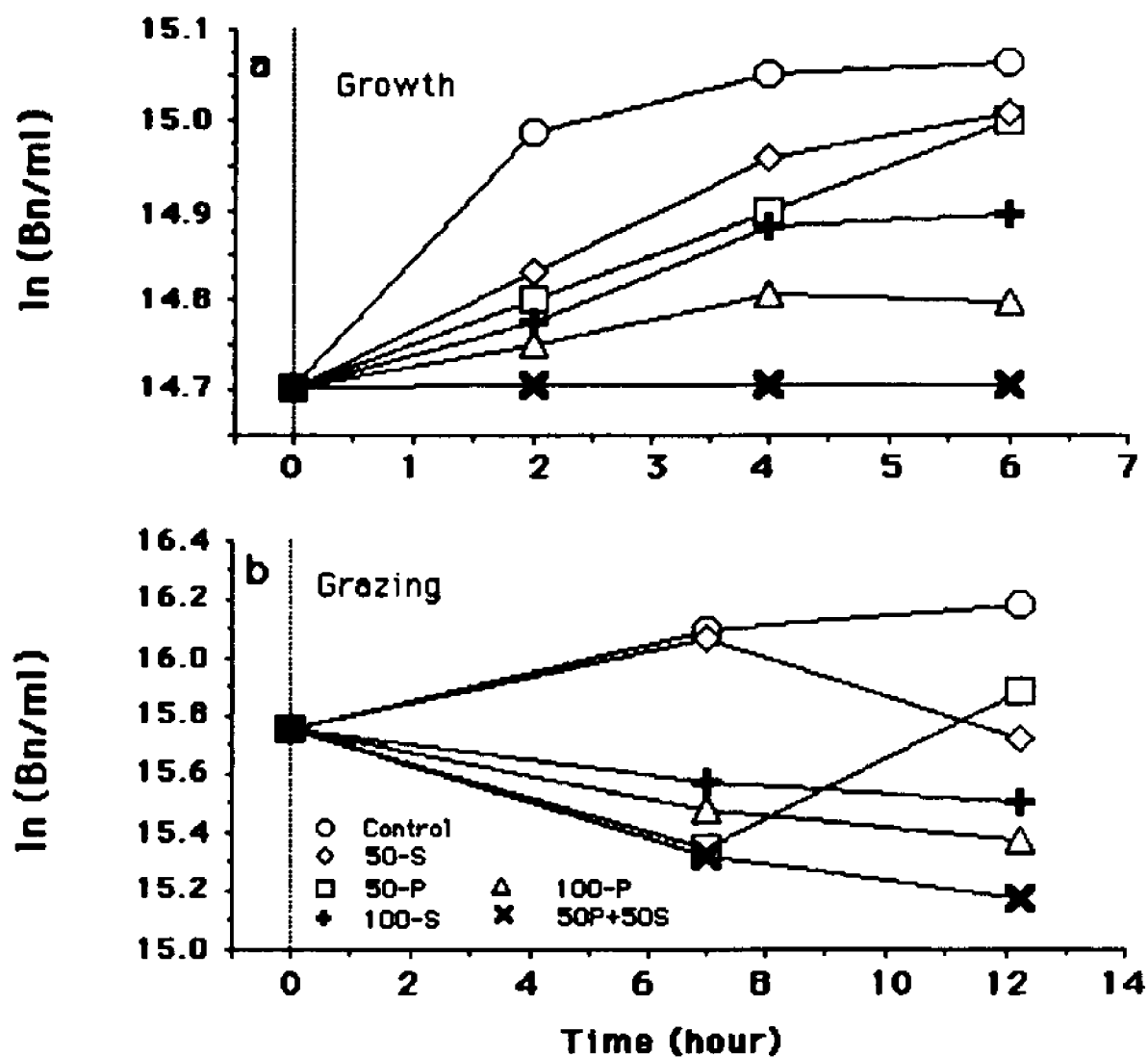


Figure 2.7. Examples of the effectiveness of penicillin and streptomycin in inhibiting bacterial growth; a: water samples were filtered through a 1.0 μm filter to separate out grazers, then the antibiotics were applied; b: the antibiotics were added into an unfiltered sample.

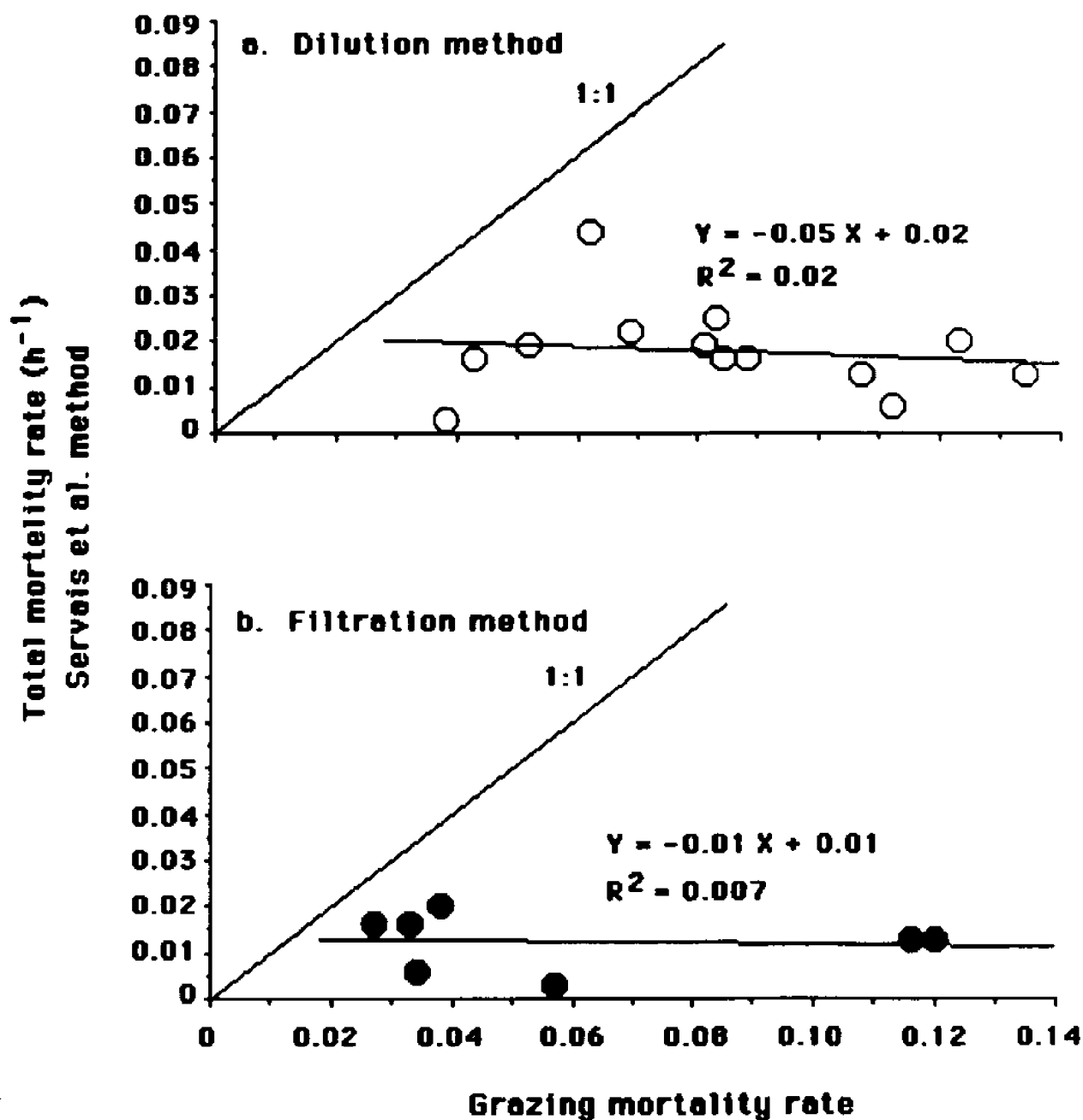


Figure 2.8. The relationship between the grazing mortality estimated using the dilution (a) and filtration (b) methods versus total mortality rates estimated using the Servais et al. (1985) method.

Table 2.1. Temperature, number of bacteria per ml, and growth and grazing mortality rates for each method during the study.

Temperature (°C)	Bn (x 10 ⁶ /ml)	Growth Rate (h ⁻¹)			Grazing Mortality Rate (h ⁻¹)			
		Dilution	Filtration	Antibiotic	Dilution	Filtration	Antibiotic	Servais
Auke Bay								
4.5	2.39	-0.0019	-0.037	*	0.095	0.048	*	*
4.5	*	-0.0092	-0.012	*	0.022	0.017	*	*
6	1.535	-0.0022	-0.018	*	0.029	0.01	*	*
9	0.508	0.006	-0.018	*	0.069	0.042	*	*
6	0.103	-0.0035	0.018	*	0.012	0.063	*	*
6	0.091	0.0078	-0.011	*	0.058	0.031	*	*
9	0.044	0.0009	0.014	*	0.026	0.038	*	*
8	0.019	0.022	0.017	*	0.033	0.039	*	*
8.5	0.046	0.021	0.022	*	0.046	0.044	*	*
Terrebonne Bay (LUMCON)								
17	3.8	0.076	0.0047	0.023	0.061	0.0157	0.034	*
20	1.7	0.076	0.035	0.0763	0.079	0.0327	0.074	*
20.5	2.14	0.073	0.048	0.0476	0.08	0.0454	0.045	*
15.25	0.808	0.027	0.0087	0.0162	0.033	0.0165	0.024	*
30.5	2.762	0.071	0.022	*	0.08	0.0257	*	*
32	3.877	0.08	0.021	*	0.098	0.035	*	*
19.2	0.579	0.078	0.013	*	0.096	0	*	*
20.3	0.699	0.06	0.012	*	0.069	0.0138	*	*
21.3	0.971	0.074	0.0016	*	0.118	0.0366	*	*
21.7	0.494	0.11	0.026	*	0.121	0.0287	*	*
18.5	0.476	0.079	0.031	*	0.114	0.045	*	*
Mississippi River								
21	1.285	0.089	0.133	0.0712	0.095	0.1292	0.075	*
20	1.001	0.093	0.07	0.015	0.08	0.05	0.035	*
18	0.76	0.079	0.105	-0.021	0.069	0.059	0.025	*
11	0.75	0.056	0.062	0.0393	0.05	0.0543	0.047	*
8	0.66	0.015	0.013	0.0205	0.018	0.0145	0.019	*
5	0.59	0.0046	0.011	0.0193	0.006	0.0123	0.018	*
2	0.57	-0.008	-0.023	-0.0251	0.019	0.003	0.0009	*
6	0.587	-0.016	-0.012	*	0.025	0.026	*	*
10	0.681	0.0088	0.03	0.05	0.038	0.057	0.023	0.003
12	0.711	0.021	0.015	0.07	0.035	0.05	0.035	*
12	1.037	0.044	0.044	-0.002	0.022	-0.008	0.05	*
21	1.044	0.071	0.157	0.0654	0.067	0.1574	0.065	*
28	1.148	0.099	0.09	0.12	0.134	0.116	0.094	0.013
28	1.148	0.076	0.098	0.111	0.107	0.12	0.089	0.013
29	1.407	0.084	0.0084	0.058	0.112	0.0344	0.032	0.006
30	2.197	0.101	0.022	0.028	0.123	0.038	0.012	0.02
27	1.664	0.051	0.01	0.067	0.084	0.033	0.044	0.016
27	1.664	0.073	0.014	0.026	0.088	0.027	0.013	0.016
Mississippi River Plume								
*	*	0.04	*	*	0.043	*	*	0.016
*	*	0.038	*	*	0.052	*	*	0.019
*	*	0.053	*	*	0.069	*	*	0.022
*	*	0.095	*	*	0.083	*	*	0.025
*	*	0.091	*	*	0.081	*	*	0.019
*	*	0.077	*	*	0.068	*	*	*
*	*	0.053	*	*	0.062	*	*	0.044

Table 2.2. The range of results obtained using various methods compared to results obtained using the dilution method using the same water sample; the numbers in brackets are the average (upper) and the number of samples (lower).

Site	Ratio to Dilution Method				
	Growth Rate		Grazing Mortality Rate		
	Filtration/ Dilution	Antibiotic/ Dilution	Filtration/ Dilution	Antibiotic/ Dilution	Servais et al./ Dilution
Auke Bay	-5.14 - 19.47 (4.09/9)	•	0.35 - 5.25 (1.29/9)	•	•
LUMCON	0.022 - 0.72 (0.31/11)	0.30 - 1.00 (0.64/4)	0.0 - 0.79 (0.36/11)	0.56 - 0.94 (0.70/4)	•
Mississippi R.	0.1 - 3.41 (1.21/18)	-0.27 - 5.68 (1.49/17)	-0.36 - 2.35 (0.90/18)	0.05 - 3.0 (0.83/17)	0.05 - 0.19 (0.13/7)
MissR. Delta Plume	•	•	•	•	0.24 - 0.71 (0.38/6)
Overall average	1.63 ± 4.3	1.33 ± 1.47	0.84 ± 0.9	0.80 ± 0.69	0.24 ± 0.18
Median	0.736	0.80	0.59	0.70	0.19
Slope	0.64 ± 0.15	0.43 ± 0.24	0.35 ± 0.16	0.35 ± 0.14	-0.05 ± 0.099

Table 2.3. Summary of major comparisons of methods and results discussed in the text.

Method	Parameter	Issue/Assumption	Result
1. Filtration	B _n	filter trapping of bacteria	size selective loss
	Growth Rate	unaffected	affected (Fuhrman and McManus, 1984); underestimates growth
	Grazing Rate	unaffected	some grazers pass through filter (Fuhrman and McManus, 1984); overestimates grazing
2. Antibiotics	Growth Rate	stops growth	almost stops growth; slightly overestimates growth
	Grazing Rate	unaffected	slightly underestimates grazing mortality
3. Servais et al.	Grazing Rate	unaffected	very low rates; greatly underestimates grazing losses
4. Dilution	Growth Rate	exponential independent of dilution	exponential independent of dilution (Tremaine and Mills, 1987)
	Grazing Rate	proportional to dilution	proportional to dilution
5. Thymidine	Growth Rate	calibrated with one of the above methods	misrepresents growth rate, per above

and Deibel, 1986), combined with significant grazing pressure and possibly bacterial lysis, might result in negative growth. Note that the grazing mortality rates were positive during these instances of negative growth. 'Negative' grazing mortality also was noted at the Mississippi River site using the filtration method, but this was the consequence of an algebraic calculation. The grazing mortality rate using the filtration method was the result of subtracting the apparent growth rate in undiluted water from the filtration growth rate; thus, the results could be either positive or negative. Negative results obtained in the growth rates estimated using the antibiotic method at the Mississippi River site can be similarly explained.

The dilution method measures growth and grazing mortality rates based on the assumption that bacterial apparent growth rates are proportional to the dilution. The filtration method measures bacterial growth rate in the absence of grazers, and measures bacterial grazing mortality caused by grazers retained on the 1 μm filter. The antibiotic method measures grazing mortality when bacteria are not growing, and the Servais et al. method measures total bacterial mortality. Since each of the methods used in this study does not measure exactly the same rate of bacterial growth or grazing mortality, it was anticipated that the results would not be equal. Since the Servais et al. method estimates grazing losses together with all other losses (e. g., lysis) it should, therefore, yield the highest loss rates. The dilution method has several advantages over the others in that it estimates both growth and grazing mortality simultaneously and uses more data to estimate the rates. The results of the dilution method were used as a standard to compare with the other methods.

Figures 2.6, 2.8, and 2.9 show the relationships between growth and grazing mortality rates estimated using the dilution method compared to all others. The statistical relationships were weak (very low R^2). Table 2.1 shows the ratio of the growth and grazing rates estimated using all other methods to the one estimated using the dilution method (for the same sampling day and location). The number of bacteria (B_n) and the

bacterial growth rate in Auke Bay were low during the study. Counting error affects the growth rate estimation, and when growth rate is low, the error in estimating the growth rate has a greater effect than if the rate were higher. This resulted in an exceptionally high ratio of growth rate estimated using the filtration method over the dilution method for some of the data. At one time this ratio reached 19.5 (Table 2.2). The average and median were used to determine the ratio. The growth rate ratios were mostly ($> 60\%$) low (< 1), but some very high ratios in Auke Bay made the average larger than one. The median ratio was closer to the majority of the ratios. Based on this discussion, the median number is preferred over the average number.

The average and median growth rates estimated using the filtration method were 1.63 ± 4.2 times and 0.74 times the dilution method, respectively. This method underestimates the growth rate by 20-30% compared to the dilution method (see discussion on filtration method). The average and median grazing mortality rate estimated using the filtration method were 0.81 ± 0.70 and 0.70 times the dilution method, respectively. This rate was the difference between the bacterial apparent growth rate in the original water sample and that estimated using the filtration method. If the growth rate was underestimated, then the grazing mortality would be overestimated.

The average and median grazing mortality rate estimated using the antibiotic method were, 0.80 ± 0.67 and 0.701 times the dilution method, respectively. This result was an underestimation (see discussion on antibiotic method) by 20 to 30% of the one estimated using the dilution method. The growth rates estimated using the antibiotic method were 1.33 ± 1.47 and 0.80 times the one estimated using the dilution method for average and median number, respectively. This rate was the difference between the bacterial apparent growth rate in the original water sample and the bacterial grazing mortality rate calculated using the antibiotic method. Because the grazing mortality was underestimated, the growth rate would be overestimated.

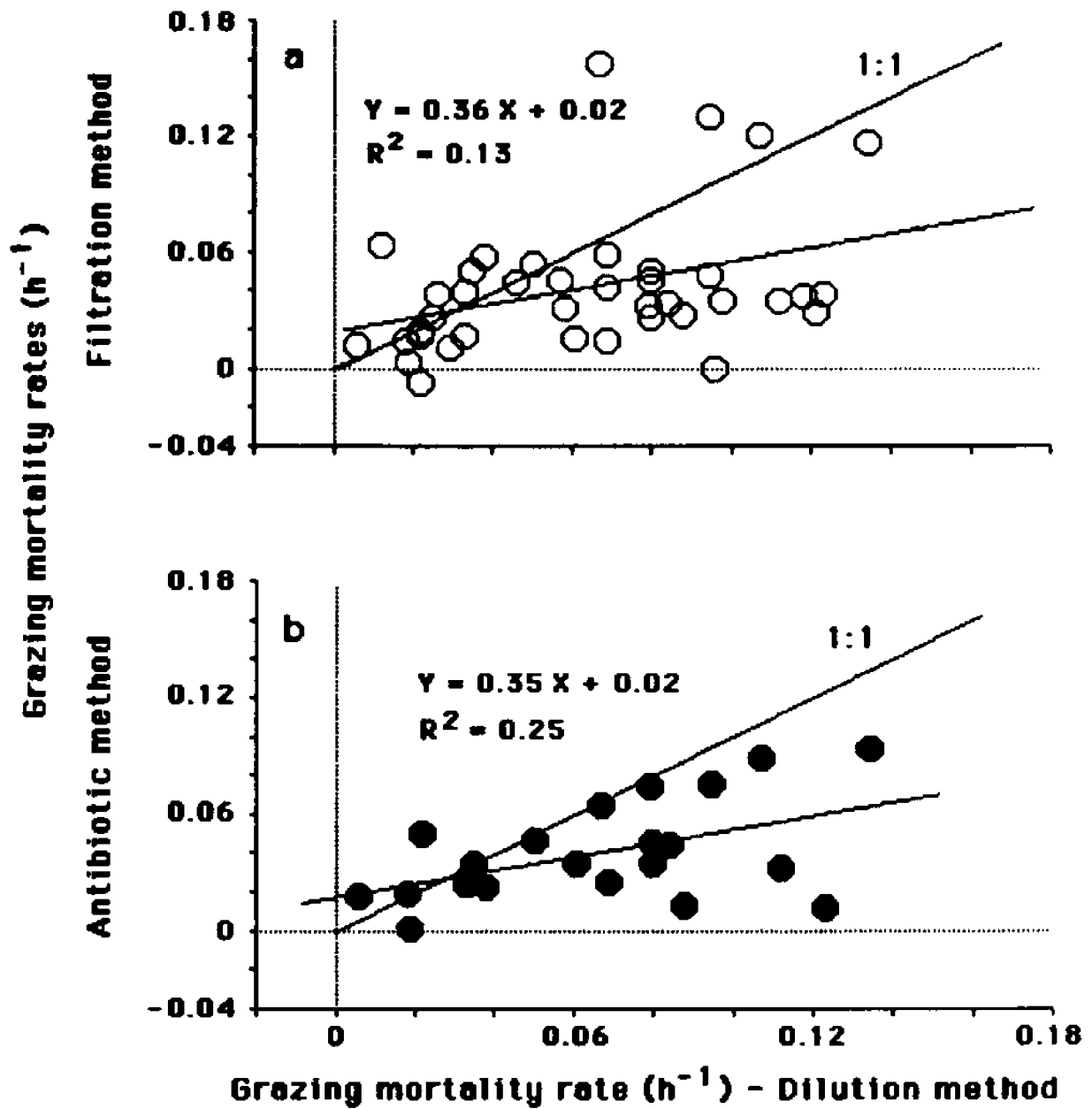


Figure 2.9. The relationship between grazing mortality rates estimated using the dilution method versus those rates estimated using the filtration (a) and antibiotic (b) methods.

The Servais et al. method provides an estimate of the total mortality, i. e., the mortality caused by both grazing and non-grazing. It should be larger than the grazing mortality alone. However, the results show that this total mortality was only about 25% (0.24 ± 0.17) of the average, and 19% of the median grazing mortality estimated using the dilution method. An apparent reason for this highly under-estimated value could be the long incubation (up to 120 hours). Changes in the bacterial community, as well as the container effect during the experiments, may lower the total mortality rate.

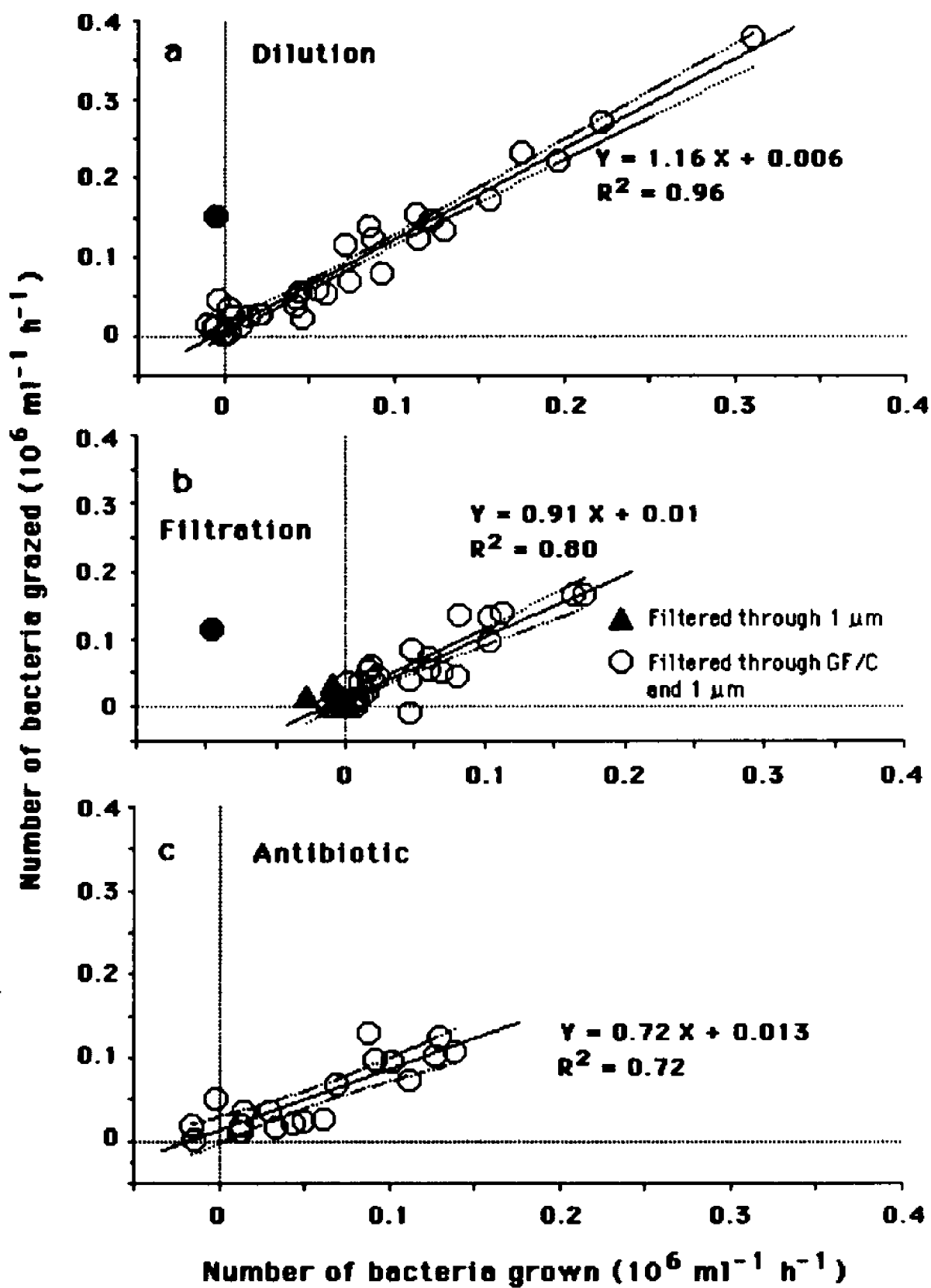
Grazing pressure.

Growth does not exactly balance grazing mortality for all methods used in this study (Table 2.1). The relationship between the Bn grazed and the Bn grown overall for the dilution, filtration, and antibiotic methods is given in Figure 2.10. The slope, intercept, and the coefficient of determination (R^2) were different among the dilution, filtration, and antibiotic methods. The difference in the grazing pressure between the dilution and filtration methods was about 25%/hr. This difference is large for bacterial dynamics because 25%/hr means the bacteria will double their number in 4 hours, or about 3 times in 12 hours. The grazing pressure difference between the dilution and antibiotic methods was even greater. Thus, in an attempt to estimate the bacterial growth and/or grazing mortality rates in the water, it is necessary to be careful in choosing a method.

Conclusions

Each method used in this study has comparative advantages and disadvantages. The Servais et al. method requires an incubation of up to 120 hours and is clinically elegant but takes the most time to process samples. The other methods used were based on temporal changes in bacterial density over 24 hours. The apparent growth rates estimated using subsamples taken three times over 24 hours give the same results as those estimated by subsampling six times over 24 hours. The dilution method is labor intensive, and needs

Figure 2.10. The relationship between the number of bacteria grown and grazed using the dilution (a), filtration (b), and antibiotic (c) methods; solid circles were excluded from the equations; the 95% confidence bands are for the true mean of Y; results from t-tests revealed that, with 95% confidence, the slopes were not different from one, but the intercepts were different from zero.



considerable time to prepare the diluent and to dilute the water samples. The filtration method requires less preparation, with the antibiotic method requiring the least amount of time.

Even though the dilution method was more labor intensive, it appears to give the best results because: 1) the filtration method underestimates bacterial growth rates and overestimates grazing mortality rates. About 50% of the bacteria are lost or trapped using this method; 2) the antibiotic method overestimates the growth rate, but underestimates grazing mortality rates; and, 3) the Servais et al. method results in an unacceptably low estimate of total mortality rate.

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CHAPTER 3

MICROBIAL GROWTH AND GRAZING MORTALITY AT THE END OF A SPRING PHYTOPLANKTON BLOOM IN AUKE BAY, ALASKA

Introduction

Bacteria are important in the marine microbial food webs because of their roles in the transformation of nutrients, accumulation of standing stocks, and consumption by predators (Azam et al., 1983; Zachary, 1978; Johannes, 1968). Most bacterial production in marine water are presumably consumed by microflagellates (Fenchel, 1982a, b). Bacterial biomass, growth rates, and grazing mortality rates in warmer climates are frequently measured. These measurements are rare in polar or cold climates, however, and the simultaneous measurement of these three parameters is even rarer.

The change of light intensity from late winter to early spring in polar climates often triggers a phytoplankton bloom. For example, within about two weeks the concentration of chlorophyll *a* may rise up to 75 times the pre-bloom concentration (e. g., Laws et al., 1988; Ziemann et al., 1990). Since phytoplankton are the major source of carbon supporting bacterial growth (Coffin and Sharp, 1987), it can be expected that the bacterial dynamics will be affected by these dramatic blooms. However, it is not clear how quickly the grazing community can respond to the rise and fall in prey density, and whether a density-dependent prey/predator relationship exists.

The purpose of this research was to study the responses of bacteria to an Arctic spring phytoplankton bloom and to determine if their growth is limited by food availability, temperature changes, or by bacterial density. Another purpose was to compare the two methods used in this study.

Materials and Methods

The sampling location was at the National Marine Fisheries Service Auke Bay Lab dock at Auke Bay, a semi-enclosed embayment in southeastern Alaska ($58^{\circ} 22' \text{ N}$; $134^{\circ} 40' \text{ W}$). The mean depth of the bay is 60 m (Laws, et al., 1988), water depth at the dock is 6 m at low tide and 13 m at the highest tide.

Samples were collected daily from April 9 to May 3, 1990, for the bacterial cell number and chlorophyll a measurements, and biweekly for the temperature experiments. Surface water samples were collected using a 2-liter Niskin bottle (Hydro Products Model No. XRB-135) from 0 to about 30 cm, i.e., the length of the Niskin bottle. Temperature and salinity were measured immediately after sampling. Chlorophyll a was measured using a Turner Design Fluorometer.

The AO (acridine orange) epifluorescence direct microscopic technique (Hobbie, et al., 1977) was used to determine bacterial density. Two milliliters of subsamples were pipetted into 4-ml sample cups, and 0.2 ml of formaldehyde was added immediately into each cup to stop bacterial production and grazing mortality. Bacteria samples were counted within 48 hours. Subsamples were stained immediately before the enumeration with 0.2 ml of 0.1% AO for about three to four minutes. Then they were vacuum-pumped at very low pressure ($<100 \text{ mm Hg}$) onto $0.2 \mu\text{m}$ black-Nuclepore filters, and rinsed twice with distilled water. The filters were mounted onto glass slides and 20 fields per filter were observed under microscope. A Leitz microscope (Laborlux 12 equipped with PLOEMOPAK) was used for this bacterial enumeration. All liquids used in this procedure (formaldehyde, AO, and distilled water) were filtered through $0.2 \mu\text{m}$ filters.

Dilution and filtration techniques were performed to measure the bacterial growth and grazing mortality rates. The purpose of using the dilution technique was to provide conditions where there were fewer encounters between bacteria and its grazers with increased dilution (Landry and Hassett, 1982). The water sample was diluted into a final

volume of 250 ml with diluent to make up 100, 50, 20, 10, and 5% concentration of the original water samples. The diluent was prepared by filtering the water sample through 0.2 μm Nuclepore filters. The diluted water was then incubated in the dark at in situ temperature, and bacterial density was determined from subsamples at 0, 5 or 20, and 24 hours. The slope of the regression line of $\ln(\text{bacteria number})$ against time was the bacterial apparent growth rate (k). Thus, k_{100} , for instance, was the bacterial apparent growth rate of the 100% or undiluted water sample. The slope of the regression line of k of each diluted sample against the dilution factor was the grazing mortality rate, and the line's intercept with the y-axis is the growth rate of bacteria.

The filtration technique was used to monitor bacterial growth after bacterivorous protozoans were removed (Fuhrman and Azam, 1980). Water samples were filtered through 1 μm Nuclepore filter to remove the bacterivorous protozoans and 250 ml of the filtered water were incubated at in situ temperature and then subsampled for bacterial density determinations. The slope of the regression line of $\ln(\text{bacteria number})$ against time is the growth rate (G_w) of the bacteria. Grazing mortality rate = $k_{100} - G_w$.

Experiments also were conducted to measure the effects of temperature on the microbial growth and grazing mortality rates. Samples for this purpose also were taken from surface waters and prepared as mentioned before. Five sets were prepared and incubated at five different temperatures using Haake Heaters (Type E 12). The first of two experiments was initially conducted at 2, 4, 6, 8, and 12 $^{\circ}\text{C}$ (the in situ temperature was 6 $^{\circ}\text{C}$). However, the temperature setting changed to 3, 3.5, 6, 7, and 18 $^{\circ}\text{C}$ during the experiment. The second experiment was set at 4, 6, 8, 10, and 12 $^{\circ}\text{C}$ (the in situ temperature was 10 $^{\circ}\text{C}$), but changed to 3, 6, 8, 12, and 18 $^{\circ}\text{C}$ during the experiment.

The 1989 glucose uptake data of Turner (1990) was analyzed. The measurement method consisted of a 10 ml sample with ^{14}C -labeled glucose solution in a plastic bottle incubated in the dark at $\pm 1^{\circ}\text{C}$ for 30 minutes. The particulate matter was collected on a

0.22 μm Nuclepore filter, and the activity counted in a Packard Tri-Carb Scintillation Counter using internal standards for calibration.

Bacterial turnover rate was calculated by dividing the number of bacteria grazed per hour by the bacterial density (in cells per hour). Results were multiplied by the average bacterial biovolume, by the average number of bacteria in one liter, by the conversion factor (biovolume to biomass), and by 24 to give the turnover rate in $\mu\text{g C/l/day}$.

The size of the bacteria, i.e., the length (L) and width (W), was measured using an eyepiece micrometer (Nikon Company, Japan). This micrometer, calibrated with an objective micrometer (Nikon Company, Japan), had arrays of vertical figures, and the distance between two figures was 0.769 μm . The cocci bacteria were assumed to have sphere shapes, while the rods were assumed to be cylindric with half spheres on each end. Bacterial biovolumes were calculated as $\{\pi(W/2)^2(L-W) + 4\pi/3(W/2)^3\}$ for rods and cocci (cocci were when $L=W$). Measurement of the bacterial biovolume in this way resulted in a 25% lower volume than by using the photograph method (from data by Bratbak, 1985).

Results and Discussion

The maximum Chl (chlorophyll *a*) value observed during the study was around Julian day 104, at 15 $\mu\text{g/l}$, about 15 times the value of two days earlier (Table 3.1). The Bn (number of bacteria) was the highest (2.4×10^6 cells/ml) at the beginning, and was the lowest ($0.019 - 0.025 \times 10^6$ cells/ml) towards the end of the study, with several intermediate fluctuations. The highest Bn was higher than the Bn in Chesapeake Bay at 3 to 4°C, but the lowest was lower than those in Chesapeake Bay, which ranged from 0.6 to 1.5×10^6 cells/ml (Quinby, et al., 1987). The bacterial growth rates ranged between -0.0092 and 0.022 hr^{-1} (mean 0.0045) for the dilution method and between -0.037 and 0.022 hr^{-1} (mean -0.0028) for the filtration method, while the range of bacterial grazing mortality rates were 0.012 to 0.095 (mean 0.043) and 0.01 to 0.048 hr^{-1} (mean 0.037) for

Table 3.1. Temperature, chlorophyll *a*, number of bacteria, bacterial growth and grazing mortality rates, and number of bacteria grown and grazed during the study.

Julian Day	Temperature (°C)	Chlorophyll <i>a</i> (µg l ⁻¹)	No. Bacteria (10 ⁶ ml ⁻¹)	Growth or Grazing Rates (% h ⁻¹)				Bacteria Grown or Grazed (10 ⁶ ml ⁻¹ h ⁻¹)			
				Dilution		Filtration		Dilution		Filtration	
				Growth	Grazing	Growth	Grazing	Grown	Grazed	Grown	Grazed
99	4.5	.	2.39	- 0.0019	0.095	-0.037	0.048	- 0.0045	0.227	- 0.088	0.115
100	4.0	0.234	0.604
101	4.5	2.155	.	- 0.0092	0.022	- 0.012	0.017
102	5.0	7.363	0.689
103	6.0	1.580	1.535	- 0.0022	0.029	- 0.018	0.01	- 0.0033	0.045	- 0.028	0.015
104	7.0	15.085
105	7.0	1.975	1.136
106	9.0	1.257	0.508	0.006	0.069	- 0.018	0.042	0.003	0.035	- 0.0091	0.021
107	8.0	1.113	0.495
108	7.0	2.155
109	6.0	3.592	0.103	- 0.0035	0.012	0.018	0.063	- 0.00036	0.0012	0.0018	0.0065
110	6.5	4.849	0.163
111	7.0	3.053	0.224
112	7.0	5.746	0.453
113	6.0	6.016	0.091	0.0078	0.058	- 0.011	0.031	0.0007	0.0053	- 0.001	0.0028
114	8.0	1.832	0.604
115	10.0	1.113	0.410
116	9.0	1.257	0.044	0.0009	0.026	0.014	0.038	0.00004	0.0011	0.0006	0.0016
117	8.0	1.275	0.024
118	11.0	0.539	0.021
119	8.0	1.374	0.019	0.022	0.033	0.017	0.039	0.00041	0.0006	0.0003	0.0007
120
121	9.5	0.897	0.034
122	8.5	2.047	0.046	0.021	0.046	0.022	0.044	0.00096	0.0021	0.001	0.002
123	8.5	0.988	0.025

dilution and filtration methods, respectively (Table 3.1). The net growth rates ranged from -0.97 to -0.011 hr^{-1} and from -0.85 to -0.022 hr^{-1} for dilution and filtration, respectively, and rose slightly during the study period. The number of bacteria grown was negative, low at the beginning, then rose, and finally almost reached zero when the number of bacteria grown was almost equal to the number of bacteria grazed per hour.

The timing of the spring bloom

Based on the data from the previous five years (Ziemann et al., 1990) the spring phytoplankton bloom (hereafter called "the bloom") in Auke Bay was expected to occur around the third week of April, 1990, or two or three weeks after this study was started. However, it appears that the 1990 bloom was earlier than anticipated.

The changes in phytoplankton density, as represented by Chl concentration, is not coincidental with the changes in Bn (Fig. 3.1). The increase and decrease in Bn lags a few days after the rise and fall in Chl concentration. The maximum correlation between Bn and Chl was when Bn was lagged by one day (Fig. 3.2). A comparable phenomenon was noticed in Auke Bay in 1989, where the peak number of bacteria was five days later than the peak of Chl concentration (Turner, 1990). Bratbak et al. (1990) found a lag of 1-3 days between diatom concentration and bacterial number in western Norway, and McManus and Peterson (1988) reported a peak of bacterial productivity 1-2 days after primary productivity during an upwelling in the nearshore zone off Central Chile.

Figure 3.3 shows the plot of Chl for 1987, 1988, 1989, and 1990 when the 1990 study was being done. If Julian day 104 in this figure was the primary bloom peak for the 1990, it is clear that this bloom was much lower than the previous ones. However, Figure 3.1 shows that the highest Bn is found at Julian day 99, at 2.4×10^6 cells/ml, and possibly also days before. If there is a two day lag between Chl concentration and Bn, then at Julian day 97 or earlier, the Chl concentration would be higher than the one at Julian day 104, perhaps over $20 \mu\text{g/l}$ Chl, based on the observed relationship between Bn and Chl. Figure

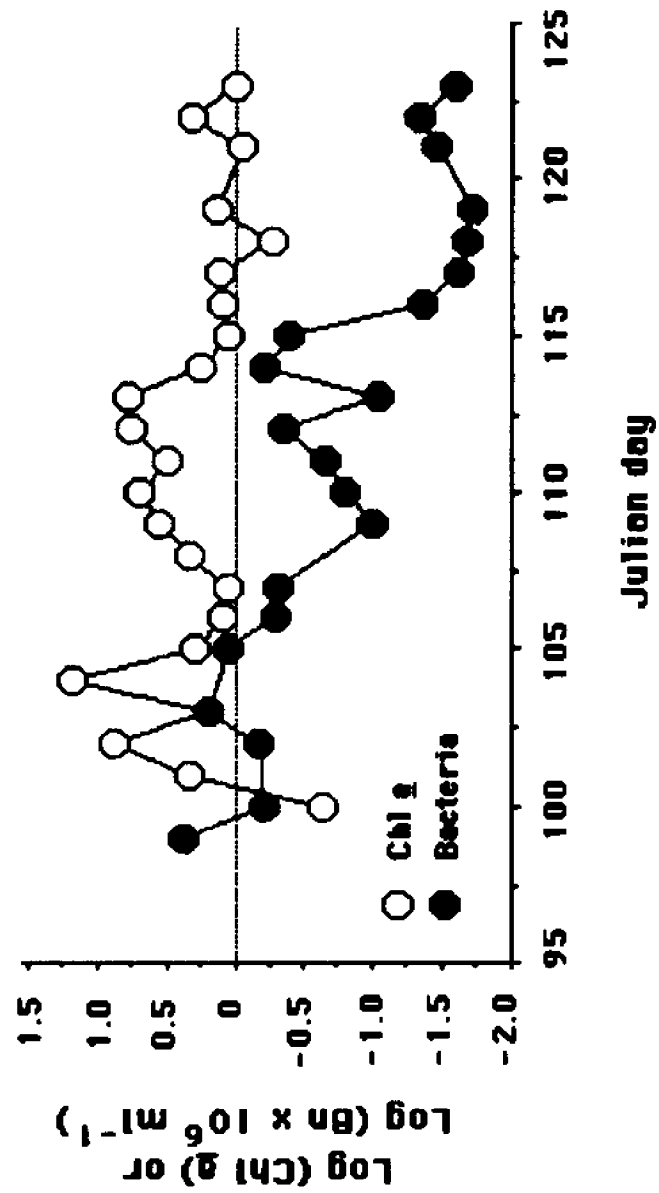


Figure 3.1. Log (Chl a) and log (Bn) during the study.

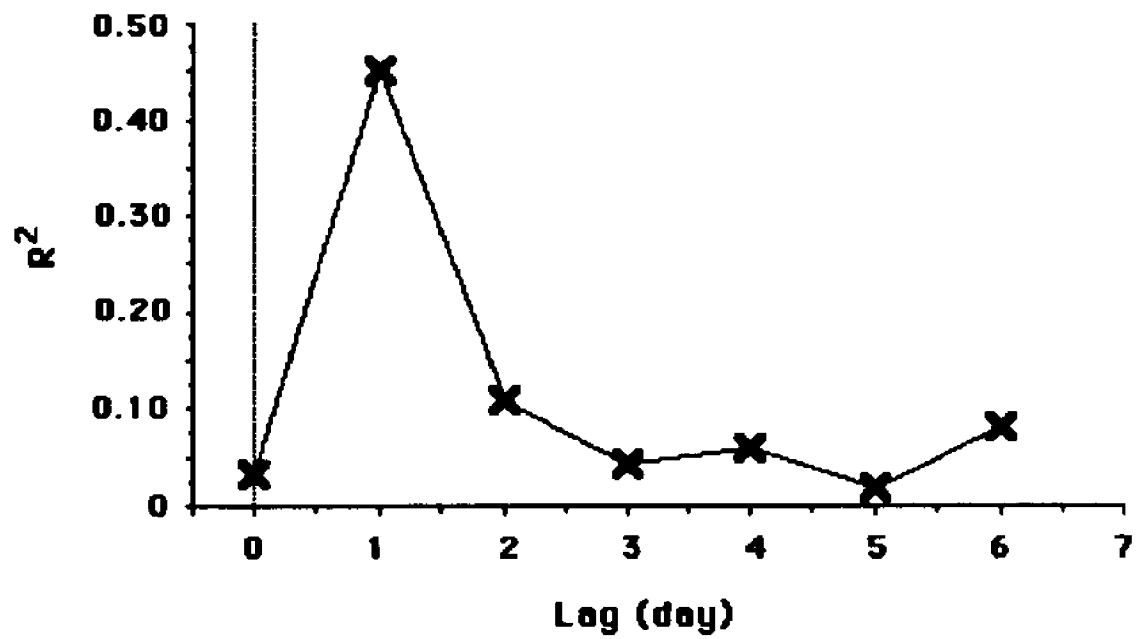


Figure 3.2. The R^2 of a linear regression of $\log(\text{Bn})$ versus $\log(\text{Chl } a)$ at different lags of $\log(\text{Bn})$.

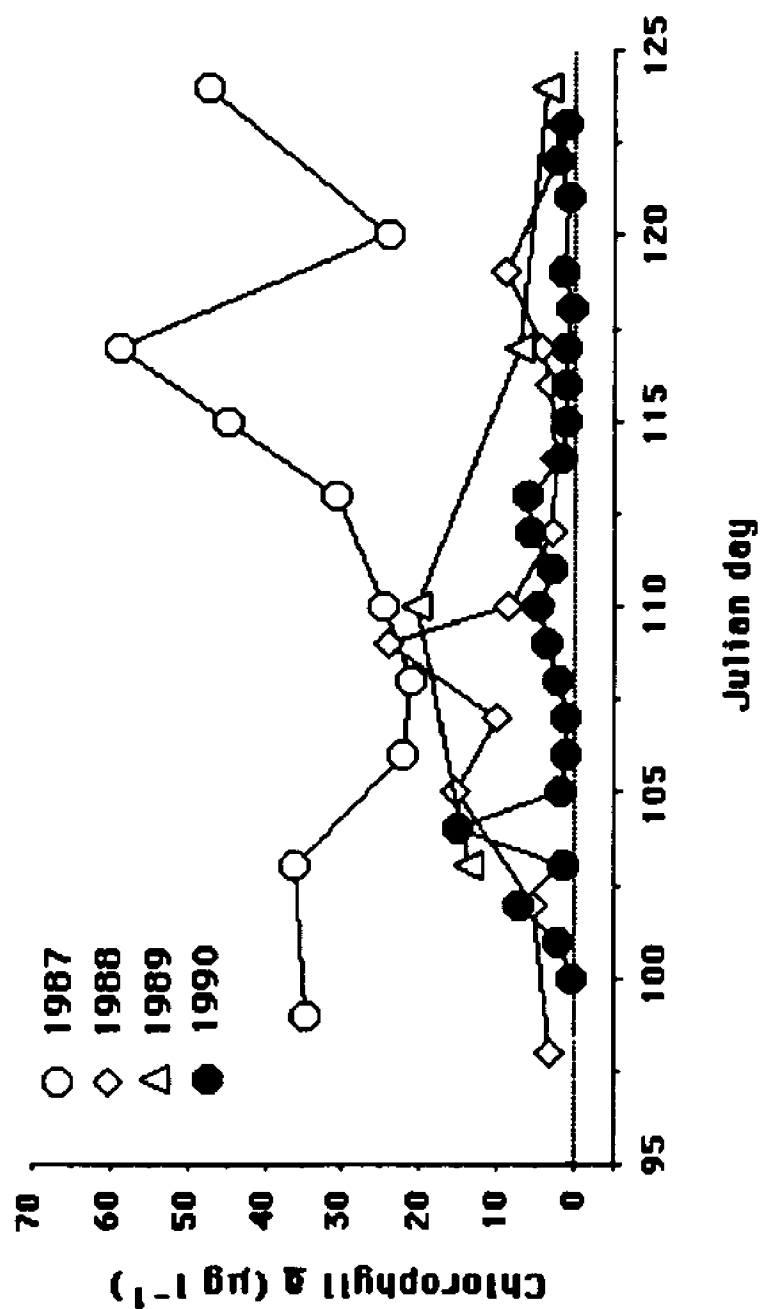


Figure 3.3. Chlorophyll a concentration (µg/l) in 1987, 1988, 1989, and 1990 during the 1990 Julian day period.

3.1 also shows that the bacterial growth was lower than its mortality (shown by the declining of bacteria cells number) during the study period, which is seemingly unlikely during a phytoplankton bloom, because the bloom should instead give good conditions for bacteria to grow rapidly. The temperatures in April were also higher in 1990 than in 1987, 1988, and 1989 (Fig. 3.4) implying an earlier development of a thermocline, water column stability and favorable light conditions. Thus, based on the above arguments concerning Bn-Chl relationship, Chl concentration, and temperature, it is concluded that this study was conducted at the end of a primary bloom.

The glucose uptake rates in Auke Bay in 1989 ranged from 0.02 to 10.8 μg glucose/l/hr, comparable to other temperate waters of similar Chl concentration. These numbers were high, indicating the capability of bacteria in the study location to absorb dissolved organics for their production.

Growth and grazing mortality rates

The observed relationships between the changes in bacterioplankton population growth and grazing mortality (hereafter "grazing") with density are: (1) the percent growth and increase in bacteria number was inversely related to Bn for both the dilution and filtration methods (Fig. 3.5 and 3.6); (2) the results using the dilution method yielded a direct relationship between Bn and both the grazing rate and number grazed, whereas there was no apparent relationship with grazing rate and number grazed, using the results from the filtration method (Fig. 3.7 and 3.8); and, (3) the statistical relationship between Bn and the number of bacteria grown or grazed was always stronger than the relationship between Bn and the rate of bacterial growth or mortality.

The direct relationship between Bn and both number grazed and mortality rate in all experiments is understandable if grazing pressure is assumed to be proportional to the concentration of prey: as prey density increases, then grazing pressure should increase.

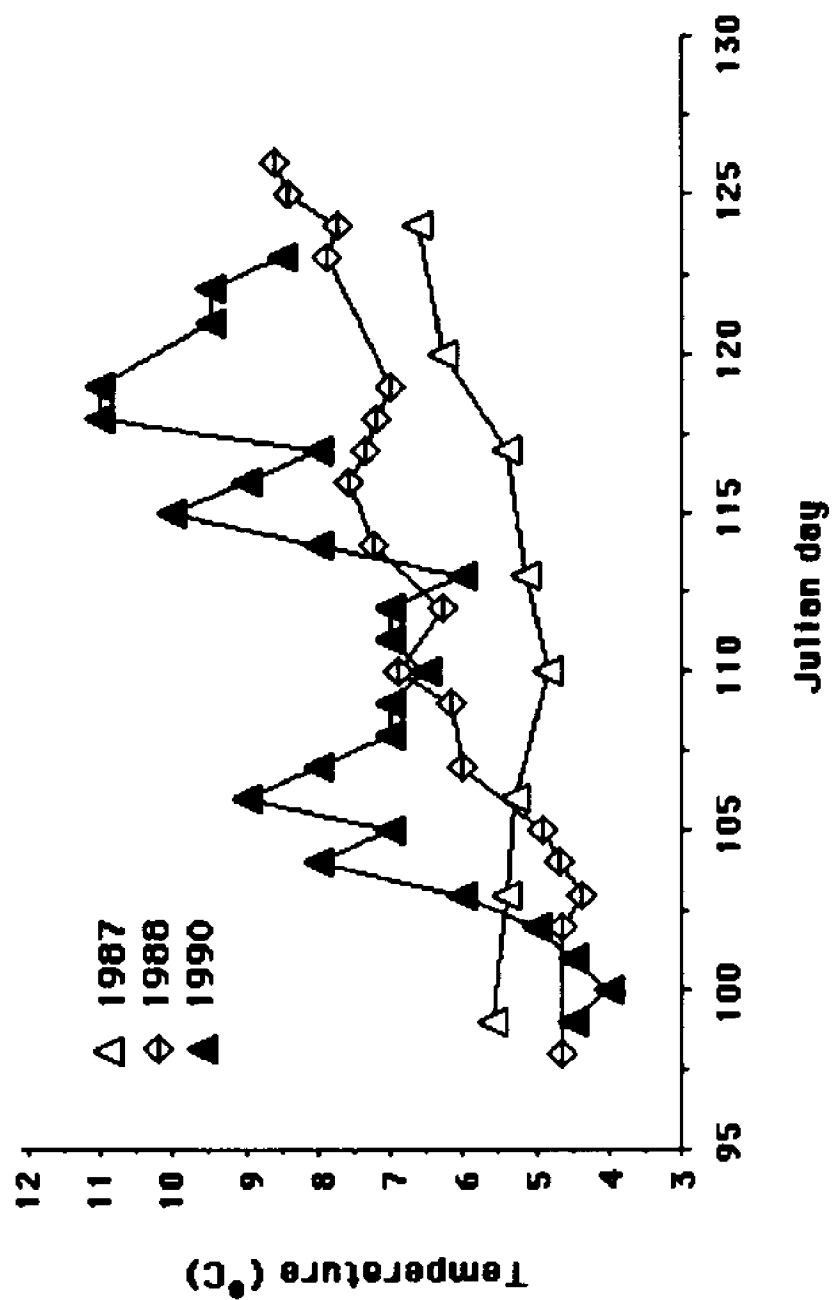


Figure 3.4. Temperature (°C) in 1987, 1988, and 1990 during the 1990 Julian day study period.

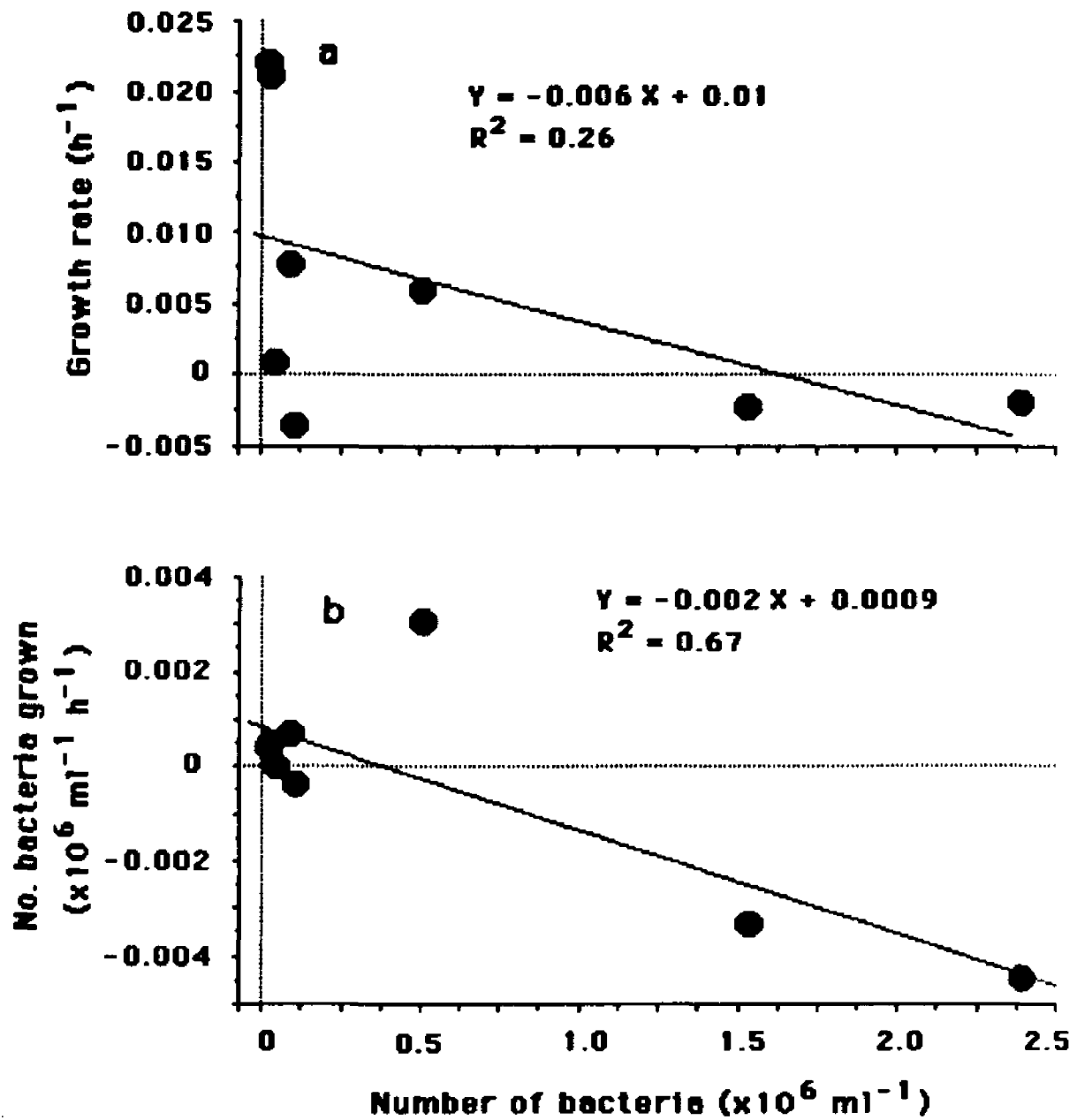


Figure 3.5. The relationships between the bacterial growth rates (a) and the the number of bacteria grown (b) versus bacterial density using the dilution method.

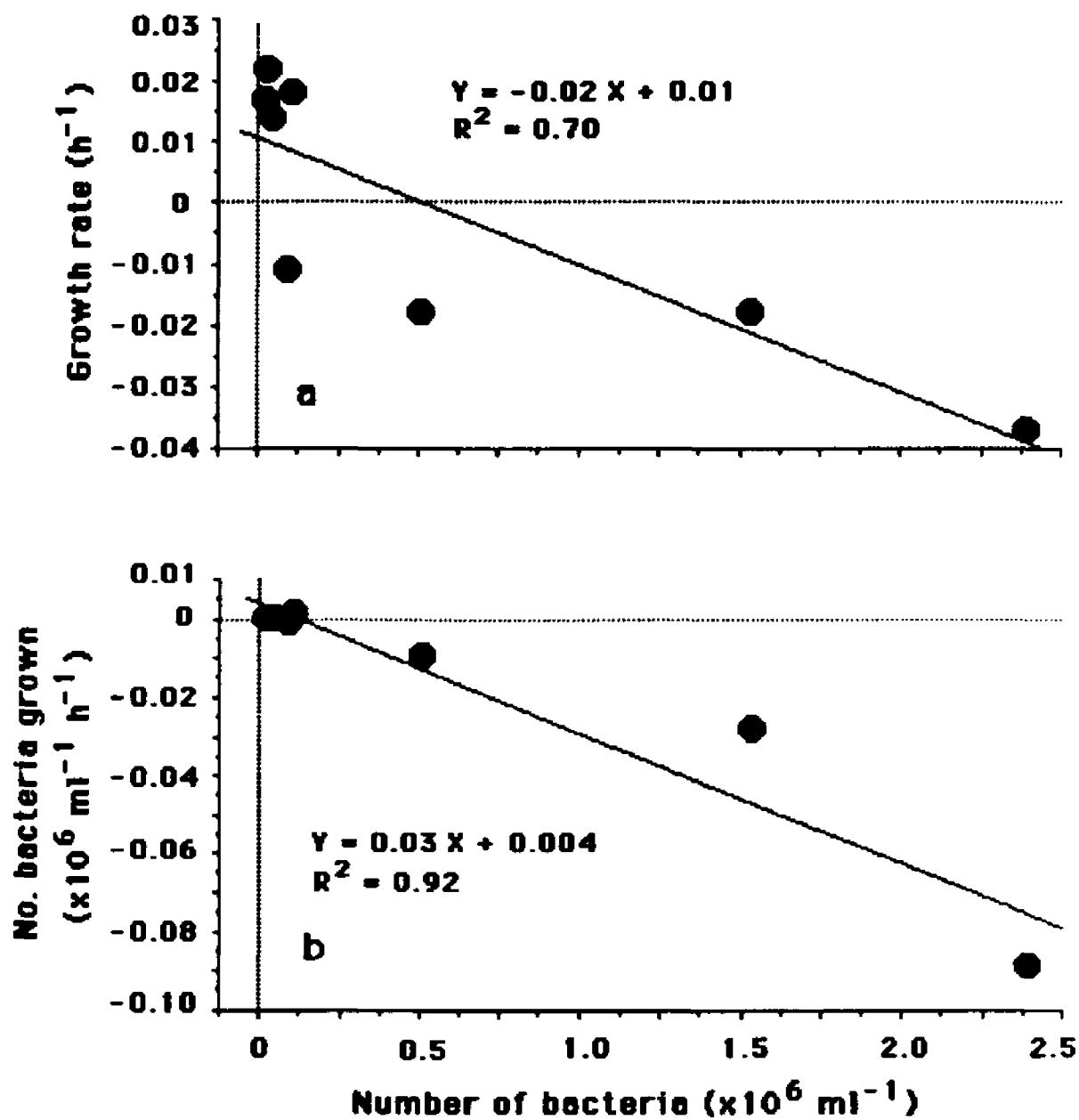


Figure 3.6. The relationships between the bacterial growth rates (a) and the number of bacteria grown (b) versus bacterial density using the filtration method.

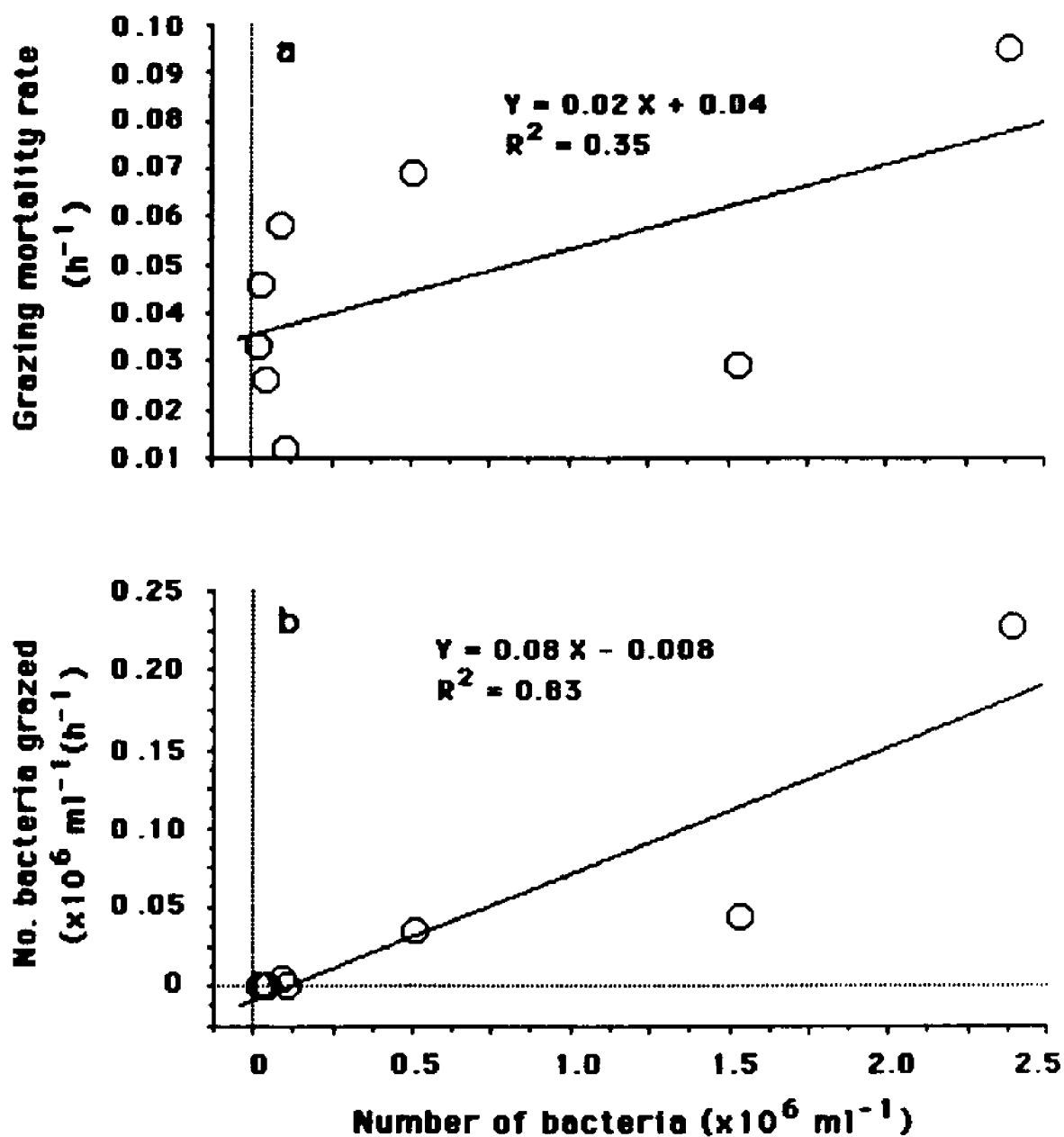


Figure 3.7. The relationships between the bacterial grazing mortality rates (a) and the number of bacteria grazed (b) versus bacterial density using the dilution method.

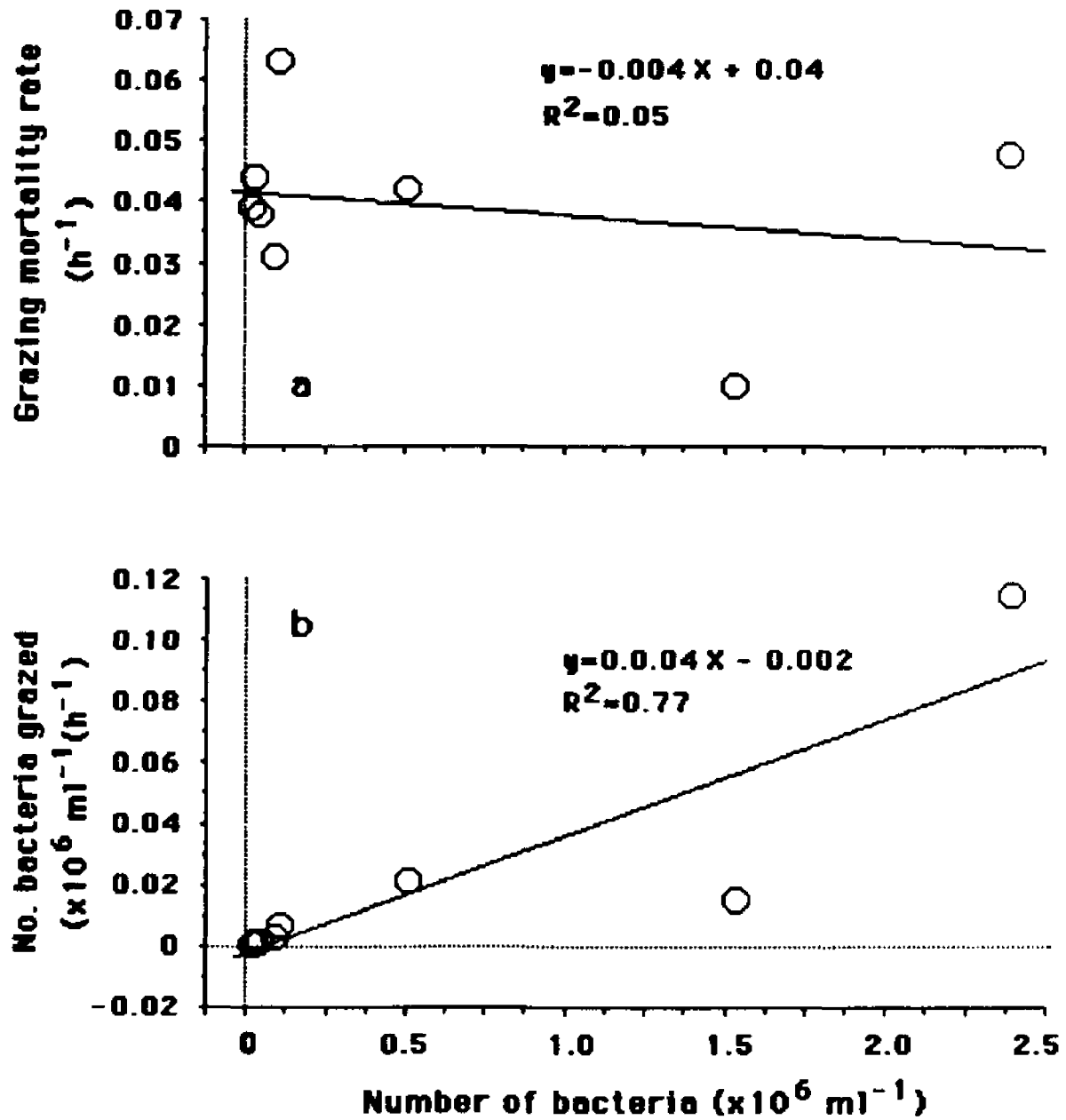


Figure 3.8. The relationships between the bacterial grazing mortality rates (a) and the number of bacteria grazed (b) versus bacterial density using the filtration method.

That is a fundamental assumption of the dilution method and has shown to be a reasonable assumption to make in interpreting the individual experiments.

The explanation for the inverse relationship between bacterial growth and B_n is not obvious but may be related to the timing of the sampling. These samples came from the end of the phytoplankton bloom, after the peak in phytoplankton production (see previous discussion). The bacterial growth rate should be constant during the period when the bacterial population is growing, but declines as predation and food resources losses accumulate. The bacterioplankton growth dropped from an average of 0.011 to -0.0025 hr^{-1} and from an average of 0.01 to -0.037 hr^{-1} for dilution and filtration methods, respectively with increasing B_n (Fig. 3.5 and 3.6). Net growth rate was negative during the first part of the sampling and approached zero as grazing rates declined towards the end of the sampling. Two explanations are postulated to explain these events, and neither is mutually exclusive: (1) competition among the bacteria with higher density and (2) food limitation.

Both growth and grazing rates varied over time, and the grazing was always higher than the growth rates (Fig. 3.9), leading to negative net growth and a decline in bacterial cell numbers during the study period (Fig. 3.1). The B_n grazed was higher than the B_n grown and tended to decrease, while the B_n grown tended to increase over time until leveling off within 2-3 weeks (Fig. 3.10). This resulted in a negative net B_n grown at the beginning, an increase in the next two or three weeks and no net growth when the B_n grown balanced the B_n grazed. This last condition is similar to a hypothetical condition at the end of a bloom (Fig. 3.11).

A weak linear regression relationship was observed between either growth or grazing rates and Chl concentration on the sampling day. However, stronger relationship were obtained between the number of bacteria grown or grazed per hour versus the Chl concentration of the previous days (Fig. 3.12).

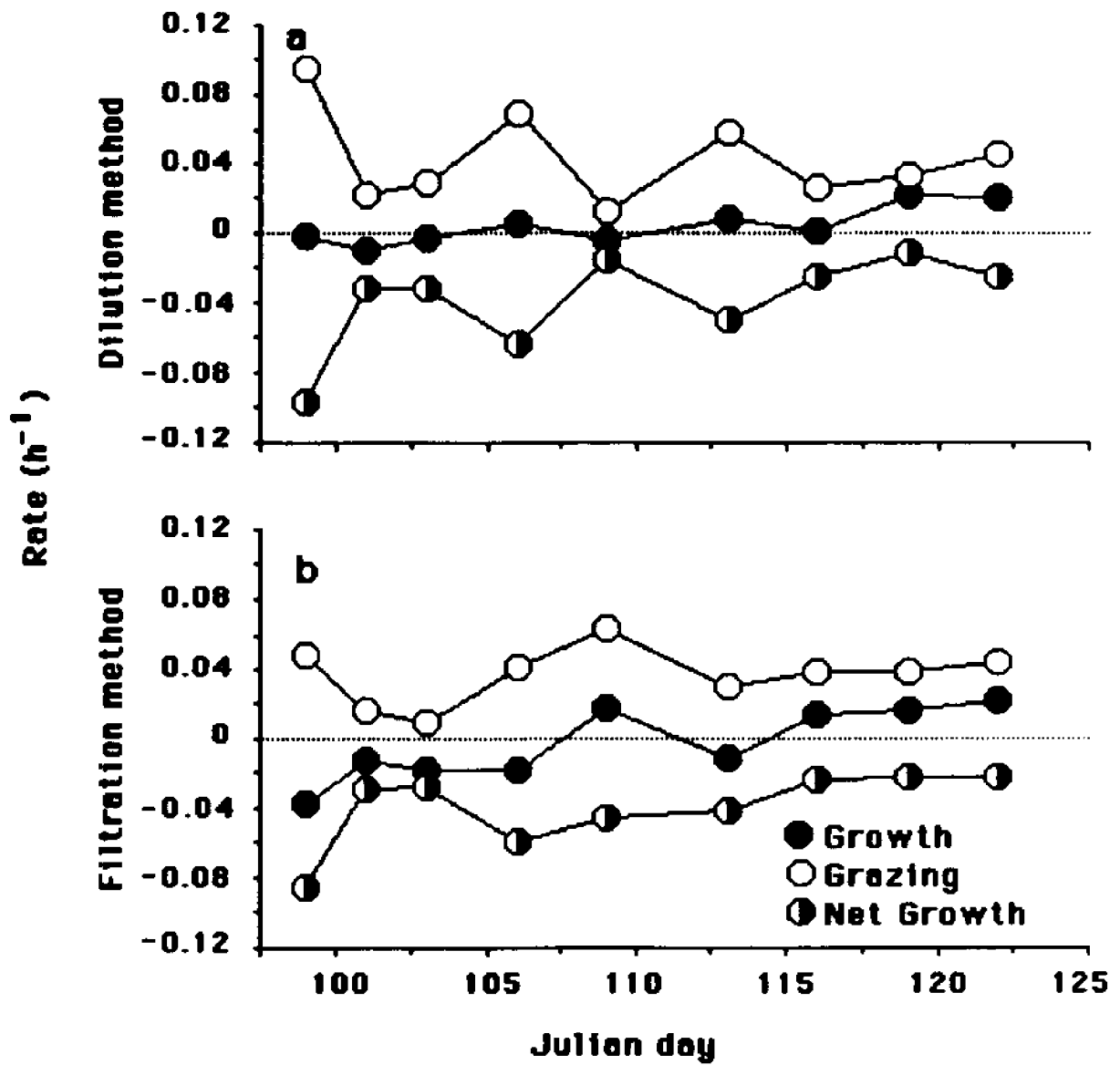


Figure 3.9. The bacterial growth, grazing mortality, and net growth rates using the dilution (a) and filtration (b) methods during the study.

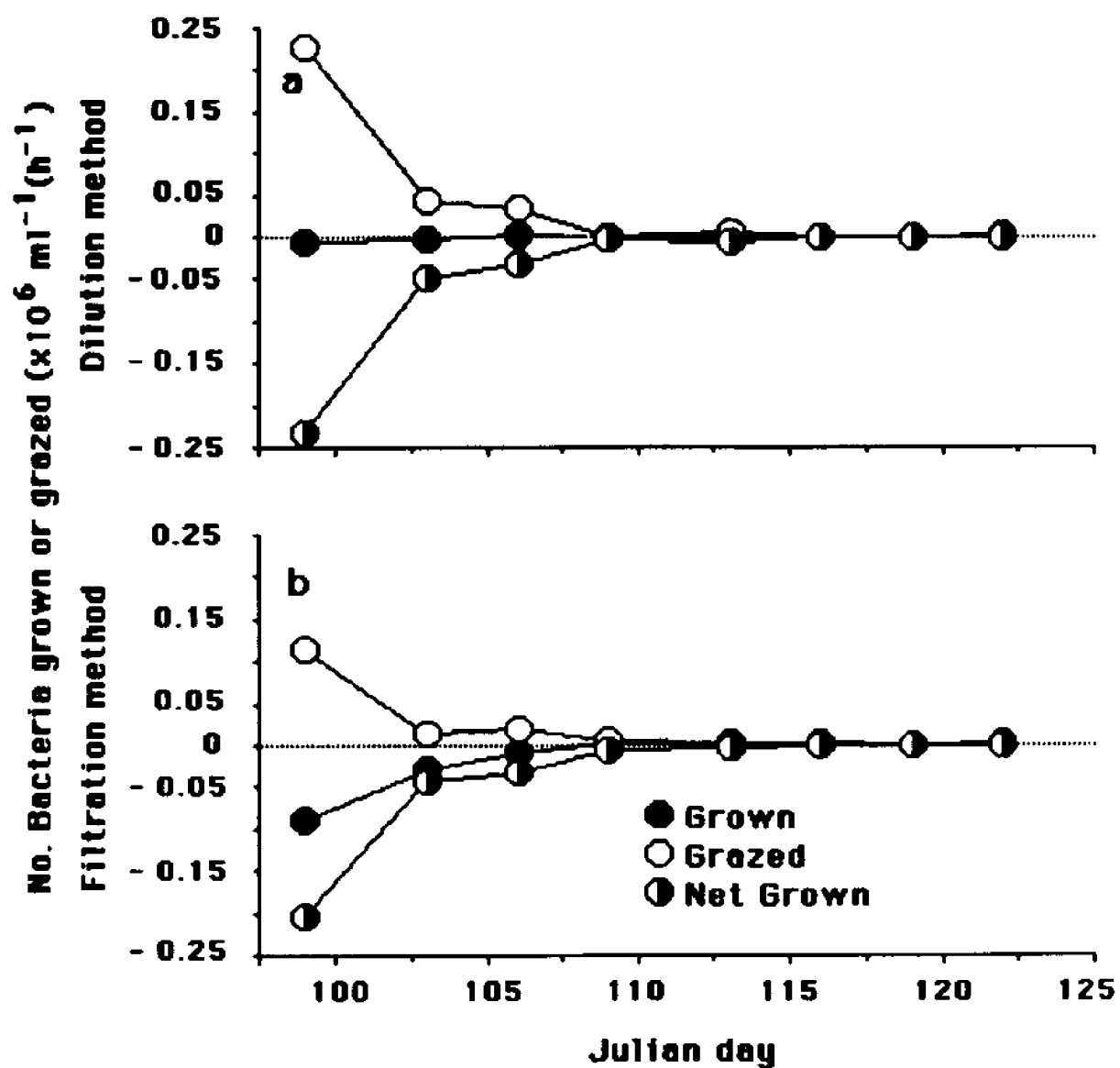


Figure 3.10. The number of bacteria grown, grazed, and net grown using the dilution (a) and filtration (b) methods versus the sampling date.

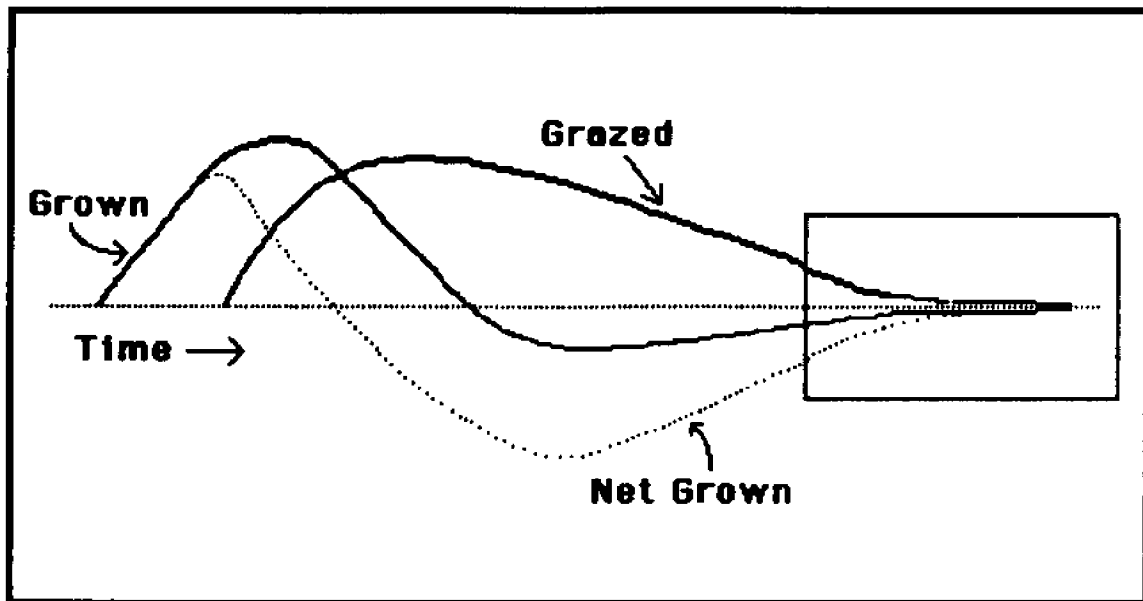


Figure 3.11. The hypothetical relationships between the number of bacteria grown, grazed, and net grown; the box at the right is the period sampled in 1990.

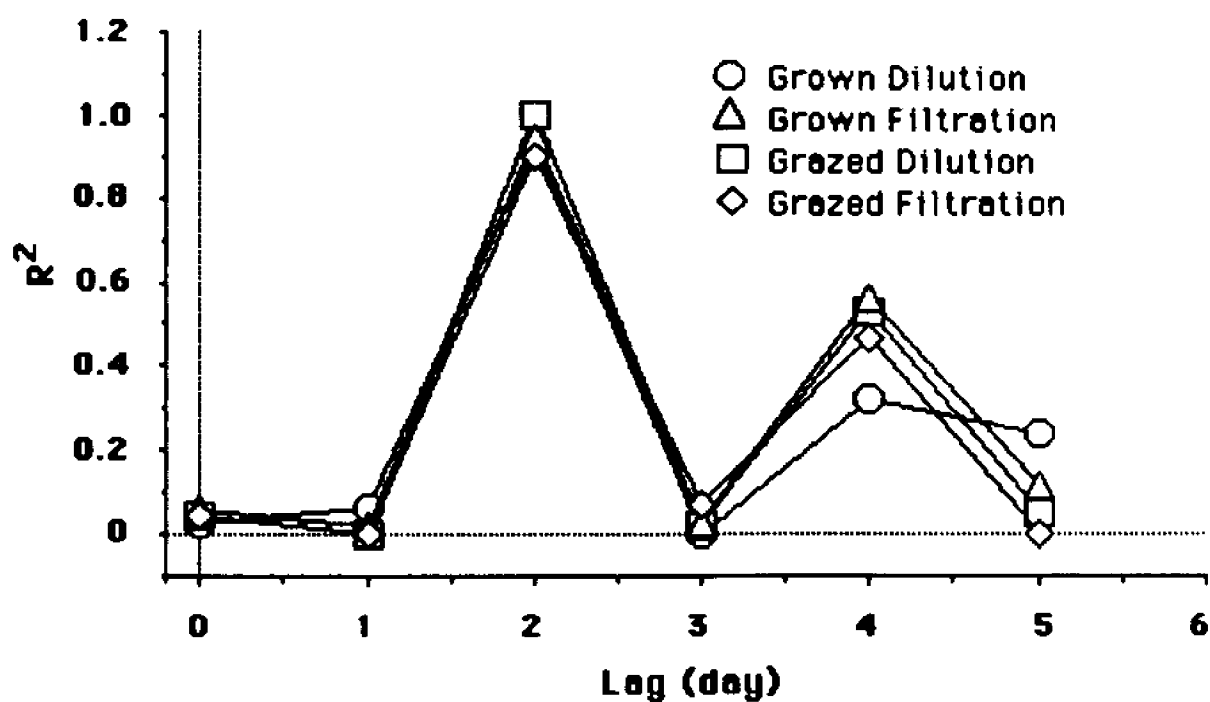


Figure 3.12. The R^2 of the number of bacteria grown and grazed versus Chl *a* (with lag) using the dilution and filtration methods.

Bacteria grew more rapidly during the decline of the Chl concentration than during its rise in 1989 and in 1990 (Fig. 3.13). A possible explanation for this result is that decaying phytoplankton release more dissolved organic carbon (food) to food-limited bacteria.

Temperature experiment

It is widely recognized that broad changes in temperature (e.g., 15° C) are a primary factor controlling bacteria in temperate coastal and estuarine waters (Vaatanen, 1980; Wilson and Stevenson, 1980; Wright and Coffin, 1983). However, the relationship between temperature and the growth, grazing, and net growth rates in this study are weak for data collected from either method in both experiments Fig. 3.14a, b). These weak relationships are especially clear between net growth rates and temperature, where there are almost no change in the rates with different temperatures.

The relationships between temperature and both the overall growth and grazing mortality rates are generally poor (Fig. 3.15a and b). Pomeroy and Deibel (1986) stated that microorganisms utilized particulate materials four to six times slower at 4° than at 20-25° C. This low activity clearly results in a low growth rate. Since the Bn is also more responsive to the Chl concentration changes, in addition to the discussions above, it appears that, at least during this study, the bacteria were limited by substrate or food, and not by temperature.

Turnover rate

The turnover rate of bacteria during the study was $4.6 \pm 2.5\%$ per hour for dilution and $3.9 \pm 1.4\%$ per hour for filtration methods, or about 1.1/day and 0.94/day for the two methods, respectively. These turnover rates were lower than 1.3 to 2.0/day observed in a coral reef community (Sorokin, 1978) but higher than 0.25 to 0.34/day found in open ocean water (Peterson, 1984) and 0.07 to 0.63/day for a warm core Gulf Stream ring

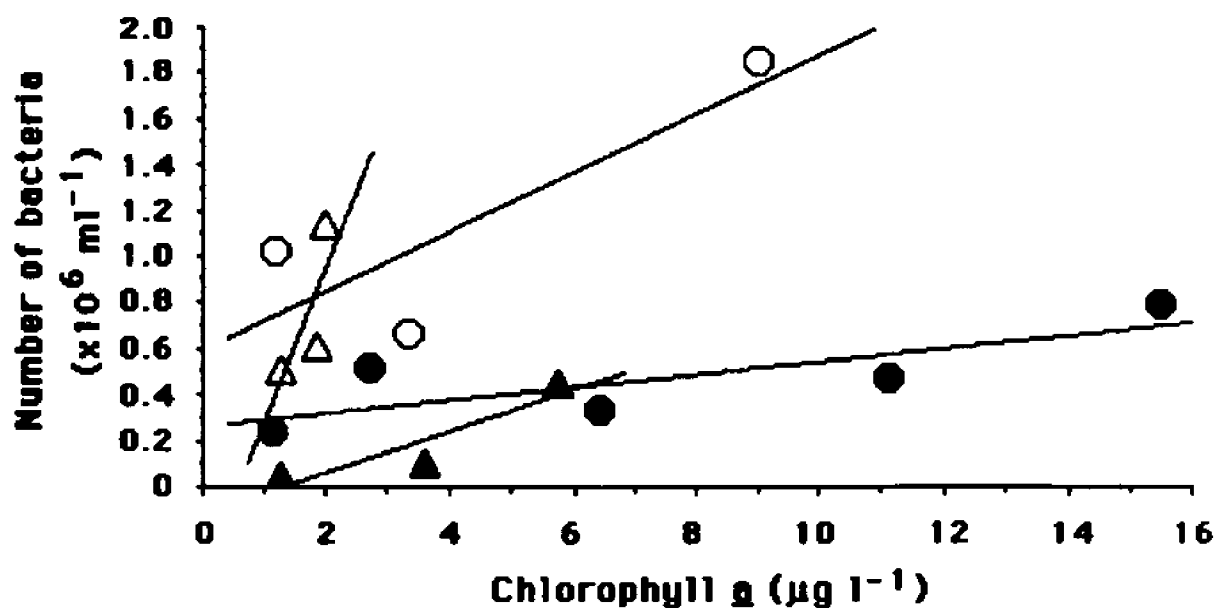


Figure 3.13. The number of bacteria during the chlorophyll rise and fall; the circles and triangles are from 1989 and 1990, respectively; solid circles or triangles are during the Chl rise, open circles or triangles are during the Chl fall; a linear regression for each data set is also shown.

Figure 3.14a. The bacterial growth, grazing mortality, and net growth rates using the dilution method during the temperature experiments.

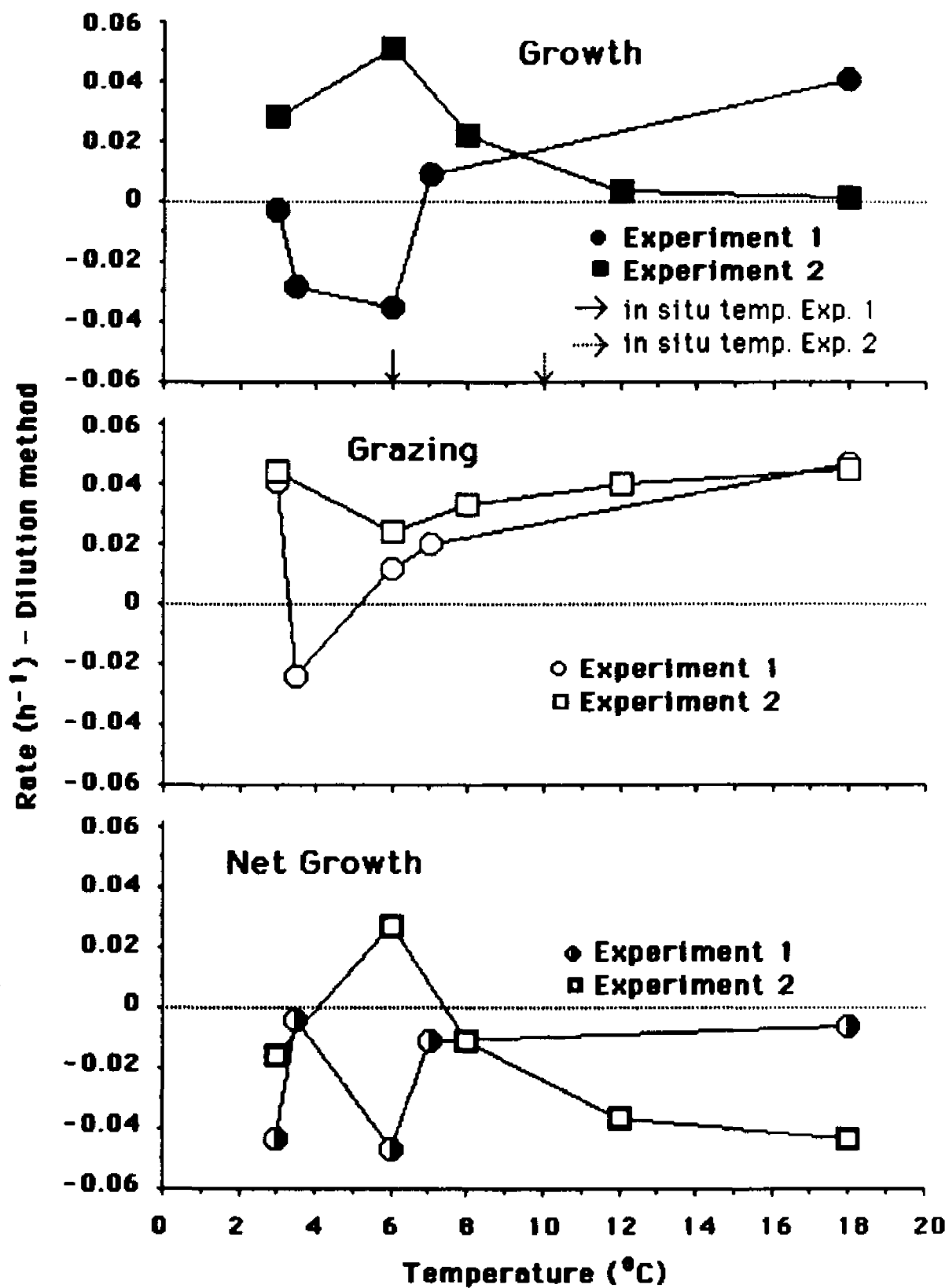
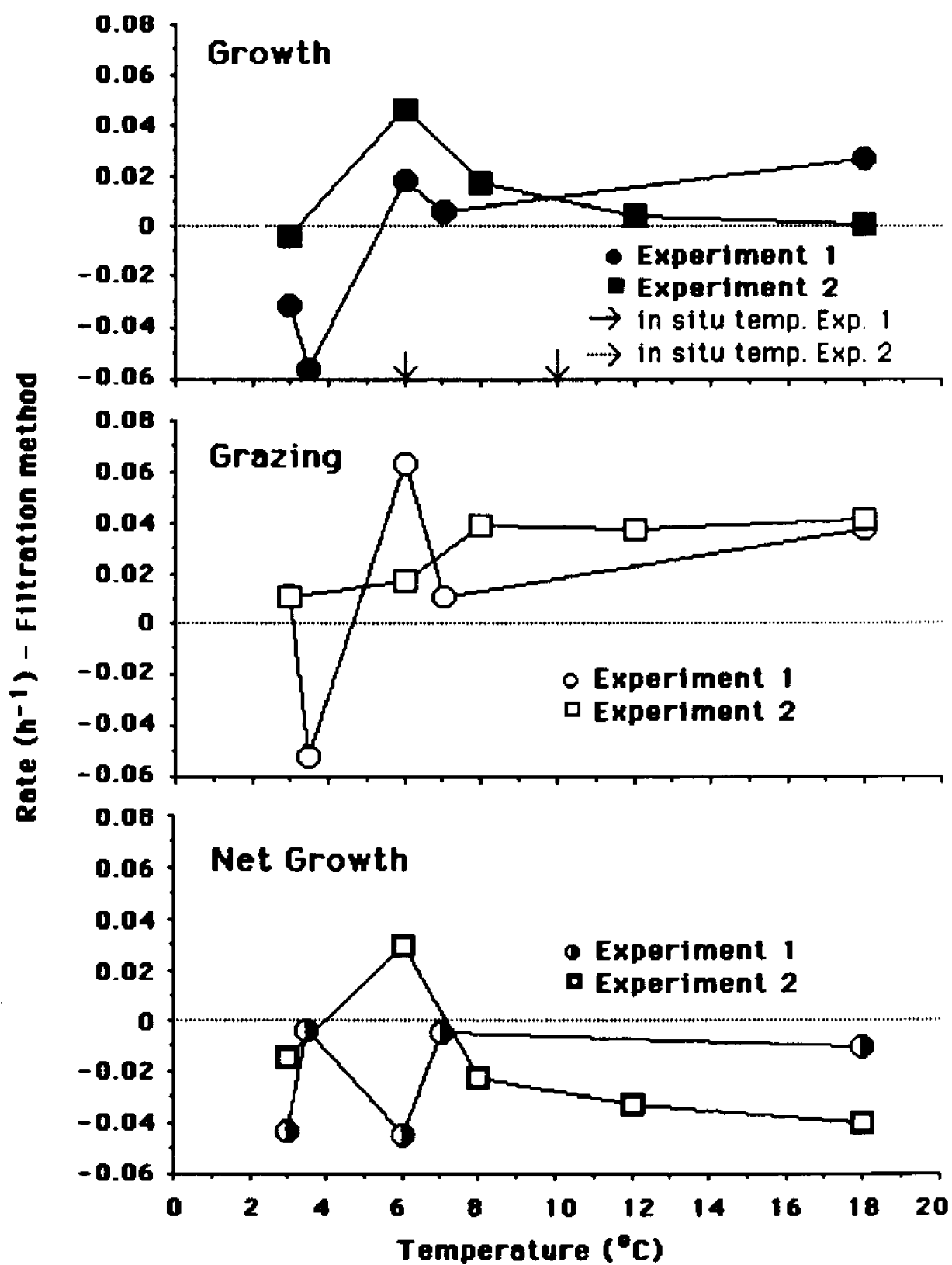


Figure 3.14b. The bacterial growth, grazing mortality, and net growth rates using the filtration method during the temperature experiments.



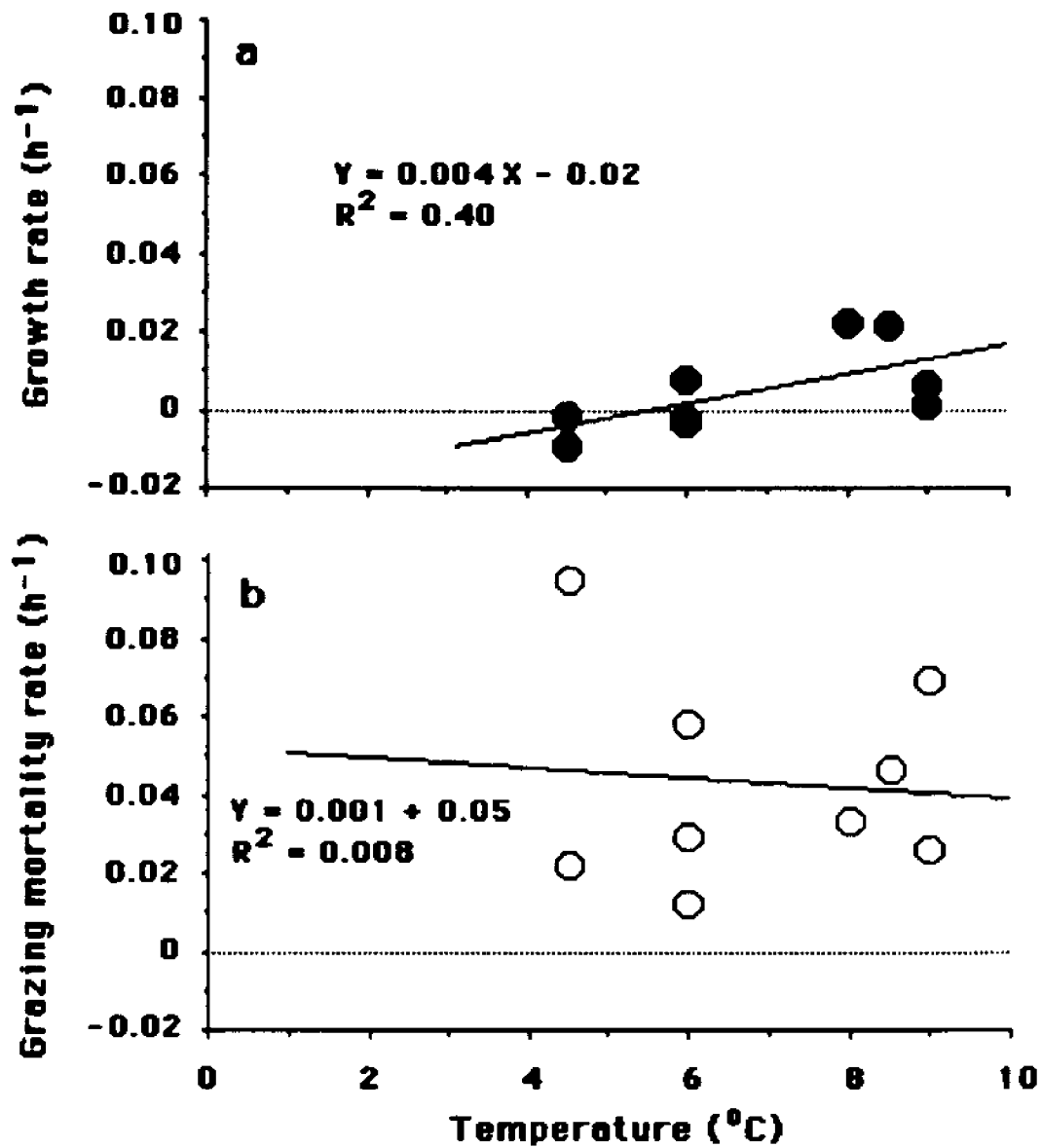


Figure 3.15a. The overall bacterial growth (a) and grazing mortality (b) rates versus temperature using the dilution method.

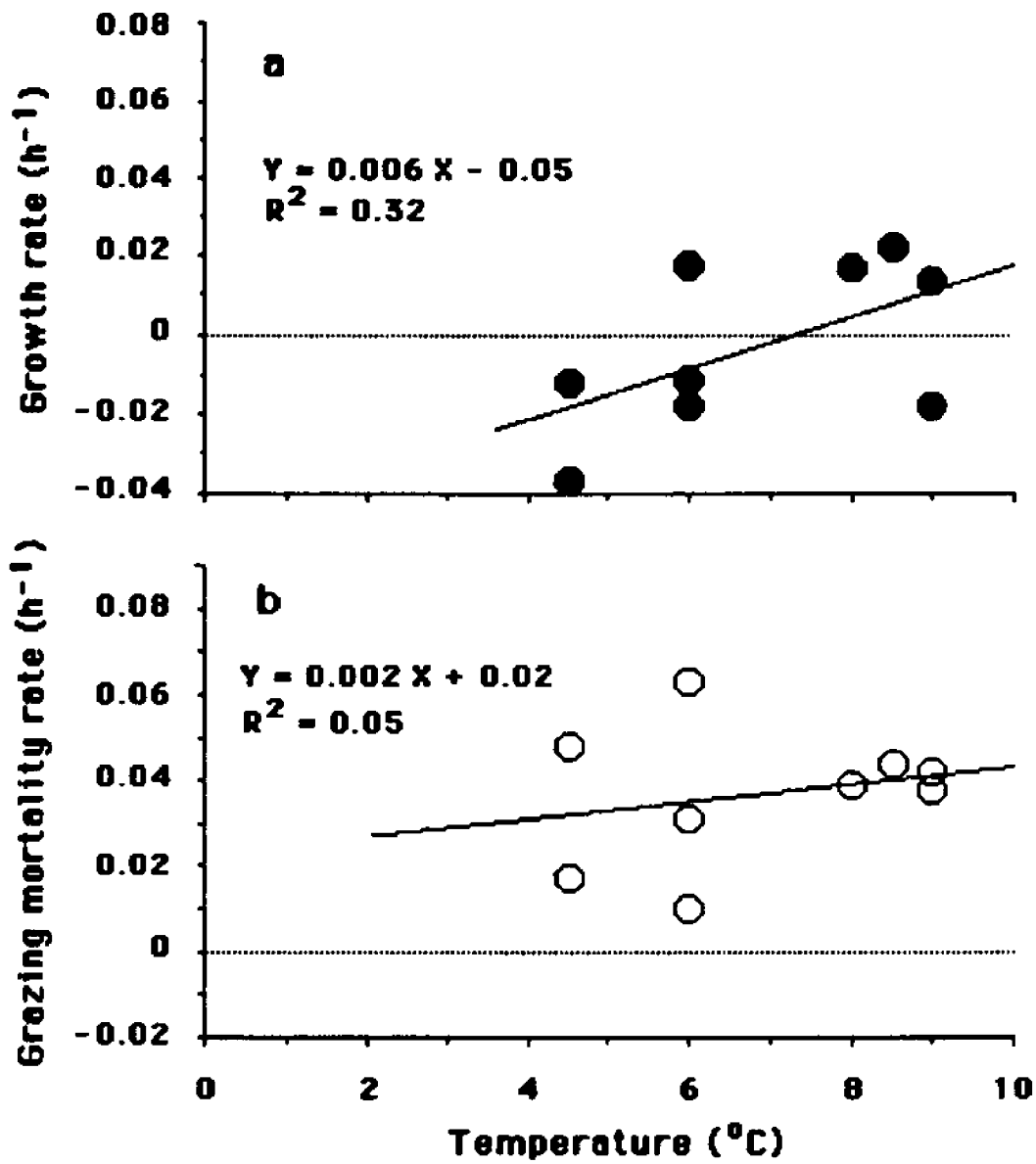


Figure 3.15b. The overall bacterial growth (a) and grazing mortality (b) rates versus temperature using the filtration method.

(Ducklow, 1986). Kogure and Koike (1987) recommended multiplying the mm^3 bacterial biovolume by 0.2 to obtain mg carbon for both pure cultured and natural bacteria from coastal and near-shore marine environments. The average bacterial biovolume and density in Auke Bay during the study was $0.234 \mu\text{m}^3/\text{cell}$ and $4.58 \times 10^8 \text{ cells/l}$, respectively. Kogure and Koike's conversion factor was used to obtain a turnover rate of $23.66 \pm 12.86 \mu\text{g C/l/day}$ for the dilution method and $20.06 \pm 7.20 \mu\text{g C/l/day}$ for using the filtration method.

Conclusions

A decreasing Chl concentration and bacteria density, the relationship between Chl and Bn, and the relatively higher temperature in April, 1990 indicate that this study was initiated at the end of a phytoplankton bloom. This conclusion is confirmed by the relationships among growth, grazing, and net growth, especially among the number of bacteria grown, grazed, and the net of the number of bacteria grown. The spring phytoplankton bloom in Auke Bay in 1990 probably occurred earlier than in the past five years.

Bacterial density changes lagged 1-2 days after the changes in Chl concentration, which is similar to the results reported by Turner (1990), Bratbak et al. (1990), and McManus and Peterson (1988). Bacteria grew more rapidly during declining Chl concentration than during a rise.

The grazing rates and the numbers of bacteria grazed were directly related to the bacteria density. No apparent relationship was noted between grazing rates and bacterial density when using the filtration method; however, an inverse relationship between numbers of bacteria grazed and bacteria density was observed. Bacterial growth rates and number of bacteria grown were inversely related to bacteria density, both for the dilution

and filtration methods. These inverse relationships may be the result of competition among the bacteria at higher density and under conditions of food limitation.

The changing water temperature in Auke Bay did not have an obvious effect on the growth and mortality rates of the bacteria during the study.

The turnover rate of bacteria was about $4.6 \pm 2.5\%/hr$ for dilution and $3.9 \pm 1.4\%/hr$ for filtration methods. Converted into carbon production, this is equivalent to a rate of $23.66 \pm 12.86 \mu g C$ and $20.06 \pm 7.20 \mu g C/l/day$, respectively. The glucose uptake rates in Auke Bay in 1989 ranged from 0.02 to $10.8 \mu g$ glucose/l/hr

The results from the dilution method were often higher than those obtained from the filtration method, but not always. The dilution method is recommended because it provides an estimate of both growth and grazing mortality rates at the same time and gives more reasonable results.

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CHAPTER 4

BACTERIOPLANKTON DYNAMICS IN THE LOWER MISSISSIPPI RIVER AT PLAQUEMINE, LOUISIANA

Introduction

Bacterioplankton dynamics have been extensively and intensively studied the last two decades in salt and fresh water, partly due to the rapid development of methodologies during those times. Observations of freshwater systems were mostly on lakes, ponds or reservoirs, and only a few on rivers (e.g., Berninger et al., 1991). Studies of large river systems, such as the Mississippi River, the largest in North America, are rare because of their size and complexity (Findlay et al., 1991).

In aquatic ecosystems, in general, phytoplankton are thought to be the major source of carbon metabolized by bacteria (Bird and Kalff, 1984; Cole et al., 1988). Bacterial abundances in clear lakes, rivers, and saltwater are reported to be well correlated with phytoplankton density (Bird and Kalff, 1984; Cole et al., 1988; Servais, 1989; Berninger et al., 1991). In Auke Bay, Alaska, bacteria were well correlated with chlorophyll concentration of 1 - 5 days earlier (Turner, 1990; Chapter 3). In contrast to the one in clear waters, bacterioplankton in turbid rivers may not be limited by in situ phytoplankton production because of light limitations on photosynthesis. Other potential controlling factors include temperature (White et al., 1991), allochthonous organic carbon (Findlay et al., 1991; Healy et al., 1988) and grazing (Wright, 1988; Gonzales et al., 1990;).

Reported here are the results of observations on surface bacterial dynamics in the lower Mississippi River at Plaquemine, Louisiana, that were intended to quantify bacterial density, growth, and grazing losses over 12 months. The hypotheses were that

temperature affected bacterial density, growth, and grazing mortality, while bacterial density was strongly correlated with bacterial growth and grazing mortality.

Materials and Methods

Water samples were taken approximately monthly using a bucket lowered from a moving ferry at about the middle of the Mississippi River at Plaquemine, Louisiana (MRP), between October 1989 and October 1990. Water temperature was measured immediately, and the sample was brought to the laboratory for experiments. The dilution method (Landry and Hassett, 1982; Chapter 2) was used in this study.

Water samples using the dilution method are diluted to a final volume of 250 ml with diluent to make up a 100, 50, 20, 10, 5, 2, and 1% solution of the original water sample. The diluent was prepared by filtering the original water sample through 0.2 μm Nuclepore filters under <100 mm Hg pressure. The various fractions were then incubated in the dark at the in situ temperature and 2 ml subsamples were taken at 0, 5, 10, 15, 20, and 24 hours. The bacteria cell numbers (N_t) in each subsample were enumerated, and the $\ln(N_t)$ regressed against time (t). Bacterial number was estimated using acridine orange direct counting method (Hobbie et al., 1977; Chapter 2). The slope of the regression line was assumed to be the apparent bacterial growth rate. This apparent bacterial growth rate is actually the result of the real growth rate minus the grazing and non-grazing mortality rates, and is represented by k_n , where n is the 100, 50, 20, 10, 5, 2, and 1% fraction of the original water sample. Thus, k_{100} was the apparent growth rate of bacteria when the density of grazers was 100% of that in the original water sample.

The sample dilutions provided a series of conditions where the encounters between bacteria and its grazers were less than in the original sample. The growth rate of bacteria when there were no grazers is k_0 , and was estimated as the intercept of the regression line of k_n and the dilution factor. The slope of the regression line was the bacterial grazing

mortality rate. Thus the dilution method is used to estimate simultaneously both bacterial growth and grazing mortality rates.

There are at least three assumptions that must be met to accept results from the dilution method: (1) the change in bacterial number over time follows an exponential model; (2) the reduction in grazing mortality is directly proportional to the dilution; and, (3) bacterial growth rates are not affected by the dilution treatment. These assumptions are discussed in the chapter on the methods comparison (Chapter 2).

An experiment also was conducted to determine the effect of altered temperature on bacterial growth rates. Water samples were incubated at 6°, 15°, and 25° C for 24 hours after being filtered with 2 µm filter to separate bacteria from their grazers (Ducklow and Hill, 1985). The change in bacterial number after incubation was considered the bacterial growth (Chapter 2).

Results

The bacterial density in the Mississippi river at the study site ranged from 0.57 to $2.2 \times 10^6 \text{ ml}^{-1}$ with the highest number observed in August 1990 (Table 4.1). The bacterial growth and grazing mortality rates ranged between - 0.008 and 0.127 h^{-1} , and between 0.006 and 0.123 h^{-1} , respectively. The estimate of the number of bacteria grown (Bn grown) and grazed (Bn grazed) during the study ranged between - 0.005 and $0.222 \times 10^6 \text{ h}^{-1}$ for bacteria grown and between 0.003 and $0.27 \times 10^6 \text{ h}^{-1}$ for bacteria grazed.

Surface water temperature in the MRP ranged from 2° to 30° C over the 12 months. A cold front arrived at the end of 1989, and the water temperature dropped to 2°-5° C between the end of December 1989 and the beginning of 1990 (Figure 4.1a and Table 4.1). The drop in water temperature during the cold front did not affect the fluctuation of the bacterial density (Fig. 4.1b) but did affect bacterial growth and grazing mortality rates

Table 4.1. Temperature, bacterial density, growth and grazing mortality rates, and the number of bacteria grown and grazed in the Mississippi River at Plaquemine during the study.

Sampling Date	Julian Day (1990)	Temperature (°C)	Bn (10 ⁶ ml ⁻¹)	Growth Rate (h ⁻¹)	Grazing Mortality Rate (h ⁻¹)	Bn Grown (10 ⁶ h ⁻¹)	Bn Grazed (10 ⁶ h ⁻¹)
10 - 05 - 1989	- 88	21	1.285	0.089	0.095	0.114	0.122
10 - 28 - 1989	- 65	20	1.001	0.093	0.08	0.093	0.08
11 - 08 - 1989	- 54	18	0.76	0.079	0.069	0.06	0.052
11 - 30 - 1989	- 32	11	0.75	0.056	0.05	0.042	0.038
12 - 12 - 1989	- 20	8	0.66	0.015	0.018	0.01	0.012
12 - 21 - 1989	- 11	5	0.59	0.005	0.006	0.003	0.004
12 - 27 - 1989	- 5	2	0.57	- 0.008	0.019	- 0.005	0.011
01 - 11 - 1990	11	6	0.587	- 0.016	0.025	- 0.009	0.015
01 - 25 - 1990	25	10	0.681	0.009	0.038	0.006	0.026
02 - 22 - 1990	53	12	0.711	0.021	0.035	0.015	0.025
03 - 11 - 1990	70	12	1.037	0.044	0.022	0.046	0.023
05 - 22 - 1990	142	21	1.044	0.071	0.067	0.074	0.07
06 - 28 - 1990	179	28	1.148	0.088	0.12	0.1	0.138
07 - 14 - 1990	195	29	1.407	0.084	0.112	0.118	0.158
08 - 28 - 1990	240	30	2.197	0.101	0.123	0.222	0.27
09 - 24 - 1990	277	27	1.664	0.062	0.086	0.103	0.143
10 - 31 - 1990	304	25	1.19	0.079	0.047	0.095	0.056

Bn = the number of bacteria

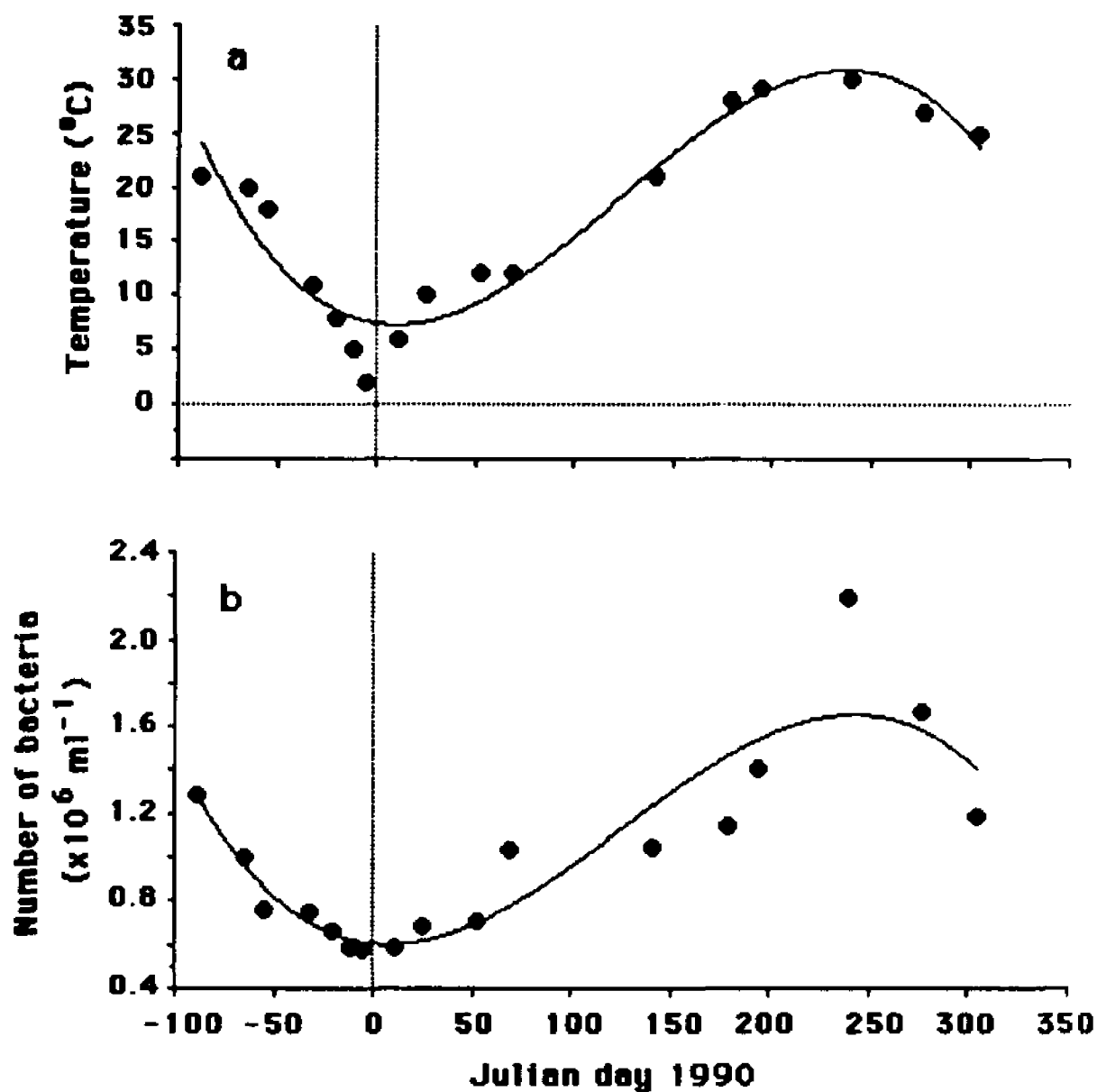


Figure 4.1. (a) Water temperature ($^{\circ}\text{C}$) fluctuations during the study; a cold front at the end of 1989 dropped the temperature to 2°C ; (b) bacterioplankton density (10^6 ml^{-1}) during the study; the cold front did not affect the density.

(Fig. 4.2a, b) and may be partly responsible for some negative bacterial growth rates observed in this study.

Overall, the bacterial density, growth rate, and grazing mortality rate were directly related to water temperature (Fig. 4.3), in contrast with observations in Auke Bay, Alaska, at the end of a spring phytoplankton bloom (Chapter 3) and in Terrebonne Bay estuary, Louisiana (Chapter 5), where the three parameters were independent of temperature. The result from the temperature experiment (Fig. 4.3b) showed that the difference in the bacterial growth rate at different temperatures was about one-tenth that observed seasonally for the river samples.

Although not as strong, especially for bacterial growth and grazing mortality rates, there were direct positive linear relationships between bacterial density and (1) growth rate, (2) grazing mortality rate, (3) Bn grown, and (4) Bn grazed (Figs. 4.4 and 4.5). The Bn grown or grazed had the stronger relationships ($R^2 = 0.90$ and 0.89 , respectively).

The linear relationship between bacterial growth and grazing mortality rates was not so strong ($R^2=0.72$, Fig. 4.6). Some grazing was observed at zero growth rates, which may explain the "negative" growth in some experiments.

The relationship between Bn grown and grazed can be separated into winter (Dec. 12 - Mar. 19), fall-spring (Sep. 23 - Dec. 11 and Mar. 20 - Jun. 20), and summer (Jun. 21 - Sep. 22) (Fig. 4.7). A linear regression between the Bn grown and grazed revealed that the growth and grazing activities were the lowest in winter and the highest in summer. In winter, the Bn grazed was 23% of the Bn grown; in combined fall and spring, the Bn grazed was 135% of the Bn grown, and in summer the Bn grazed was 108 % of the Bn grown. For the overall annual relationship, the Bn grazed was 13% higher than the Bn grown, which was not different from zero at 95% confidence intervals.

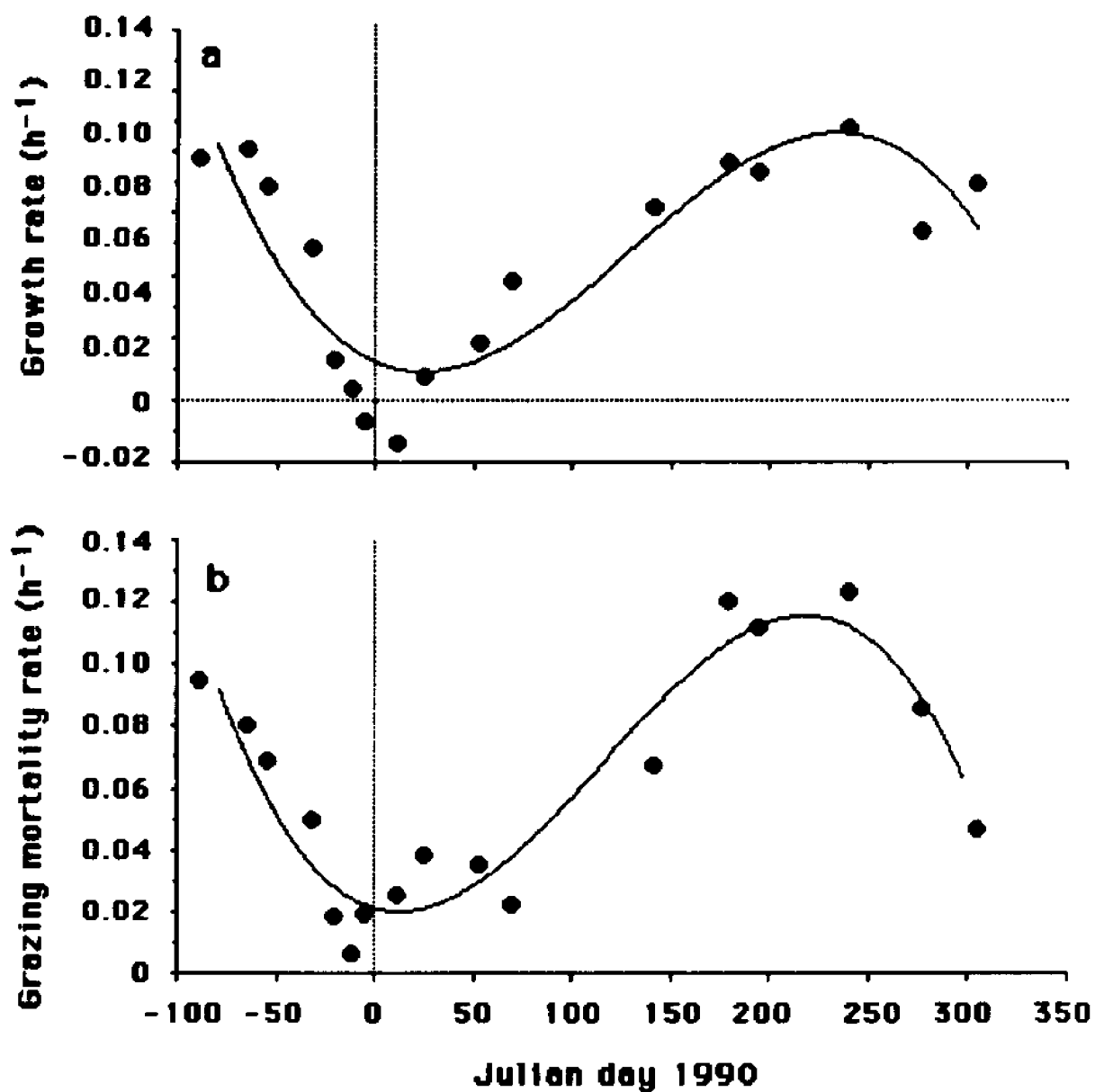
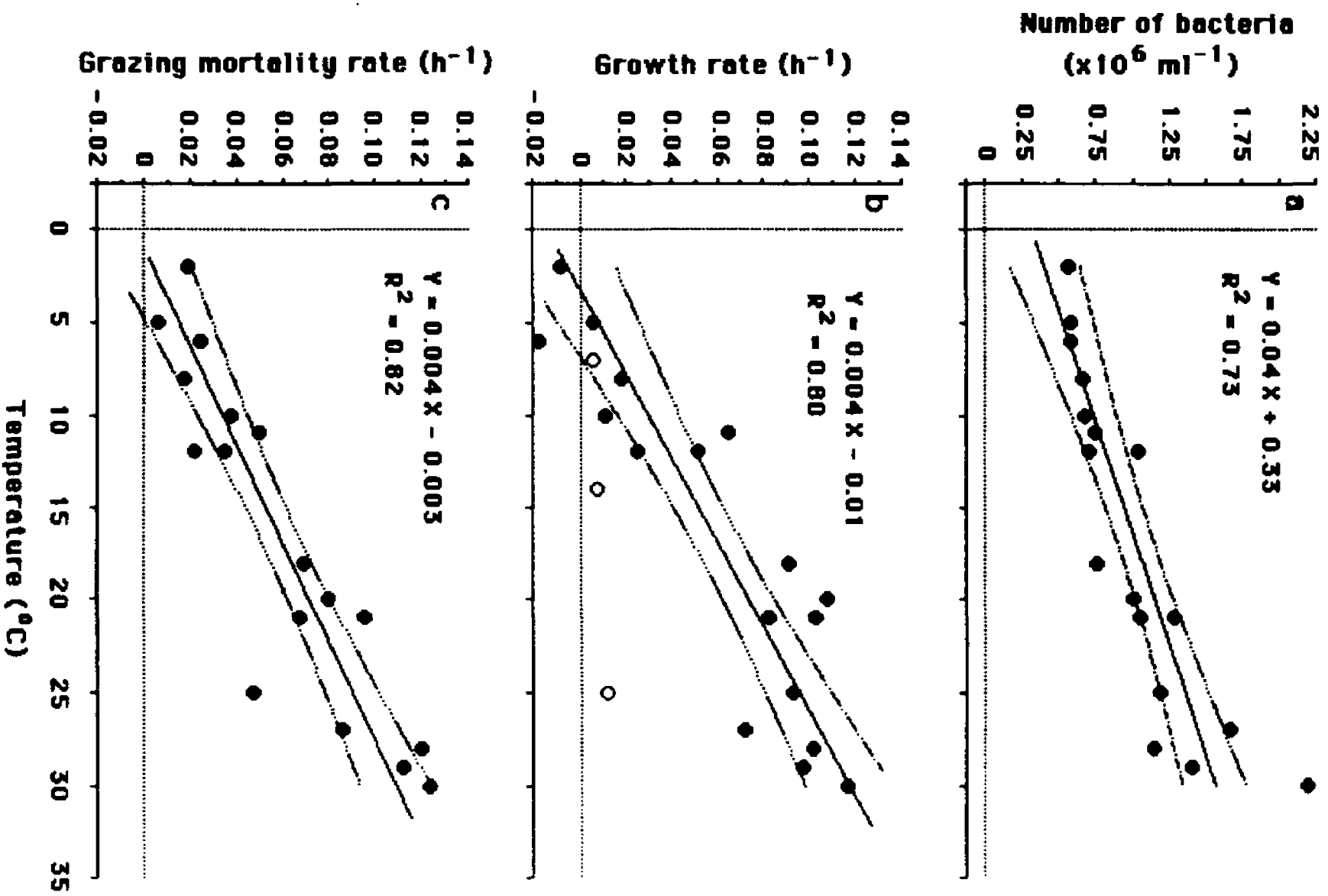


Figure 4.2. The bacterioplankton growth (a) and grazing mortality (b) rates fluctuations during the study; the cold front in 1989 clearly affected the growth and grazing mortality.

Figure 4.3. The relationships between bacterioplankton density (a), bacterial growth (b) and grazing mortality (c) rates versus water temperature; the open circles in 4.3b are results from the temperature experiment; the curved lines are the 95% confidence bands for the true mean of Y.



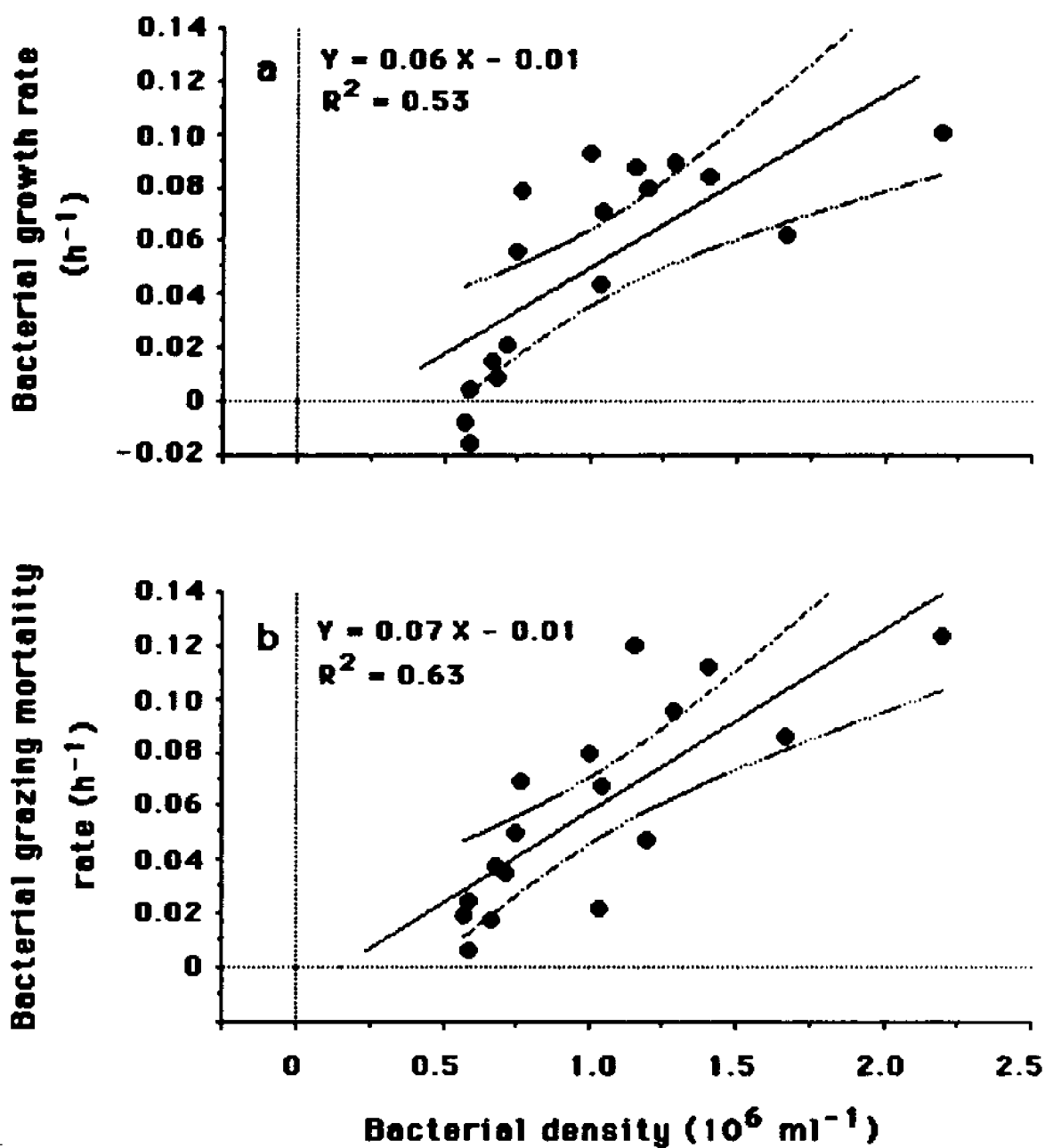


Figure 4.4. The relationships between bacterial density versus bacterial growth (a) and grazing mortality (b) rates; the curved lines are the 95% confidence bands for the true mean of Y.

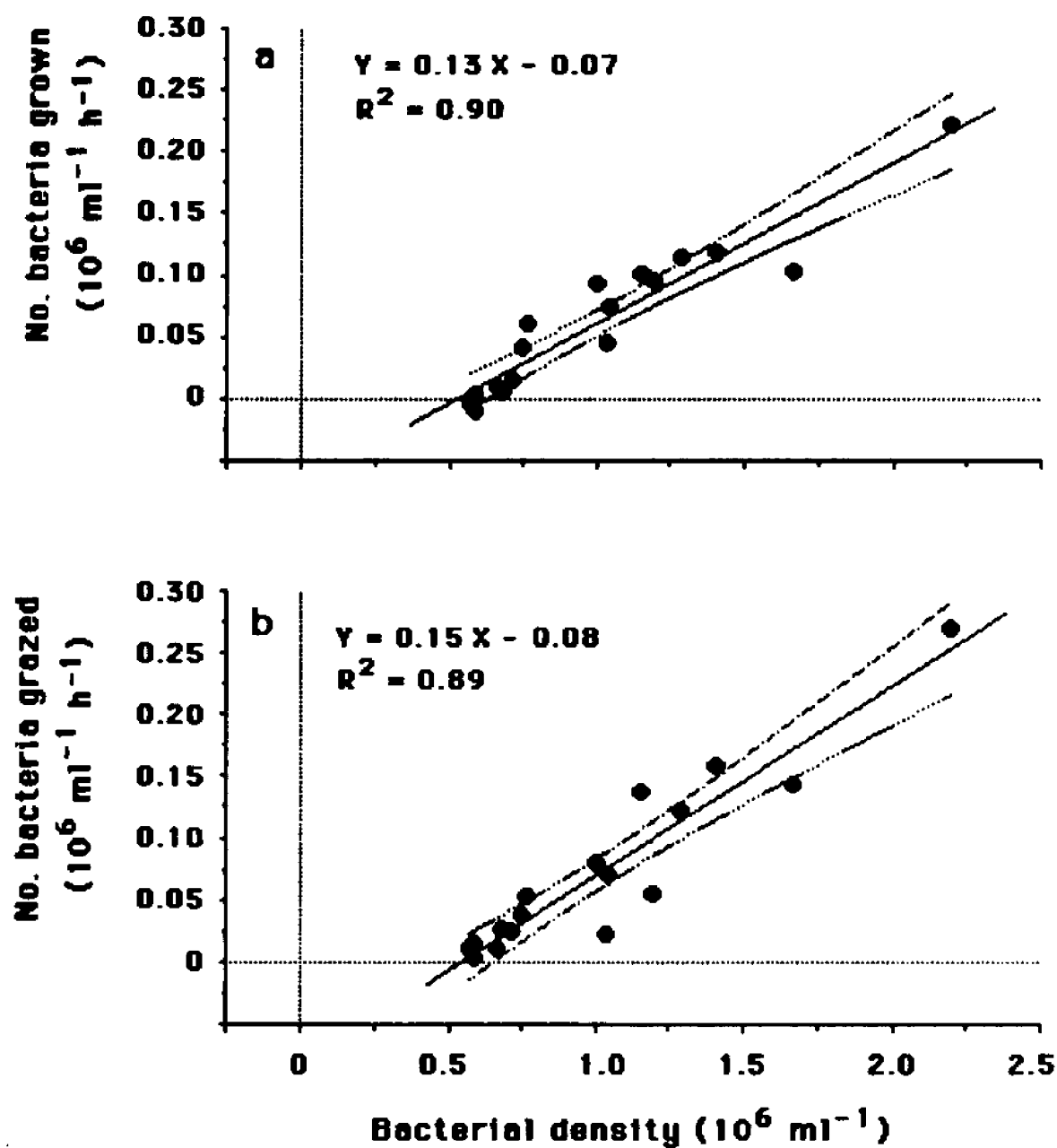


Figure 4.5. The relationships between bacterial density and the number of bacteria grown (a) and grazed (b); about 13% and 15% of the bacterial population were added and taken up every hour, respectively, to and from the ecosystem; the curved lines are the 95% confidence bands for the true mean of Y.

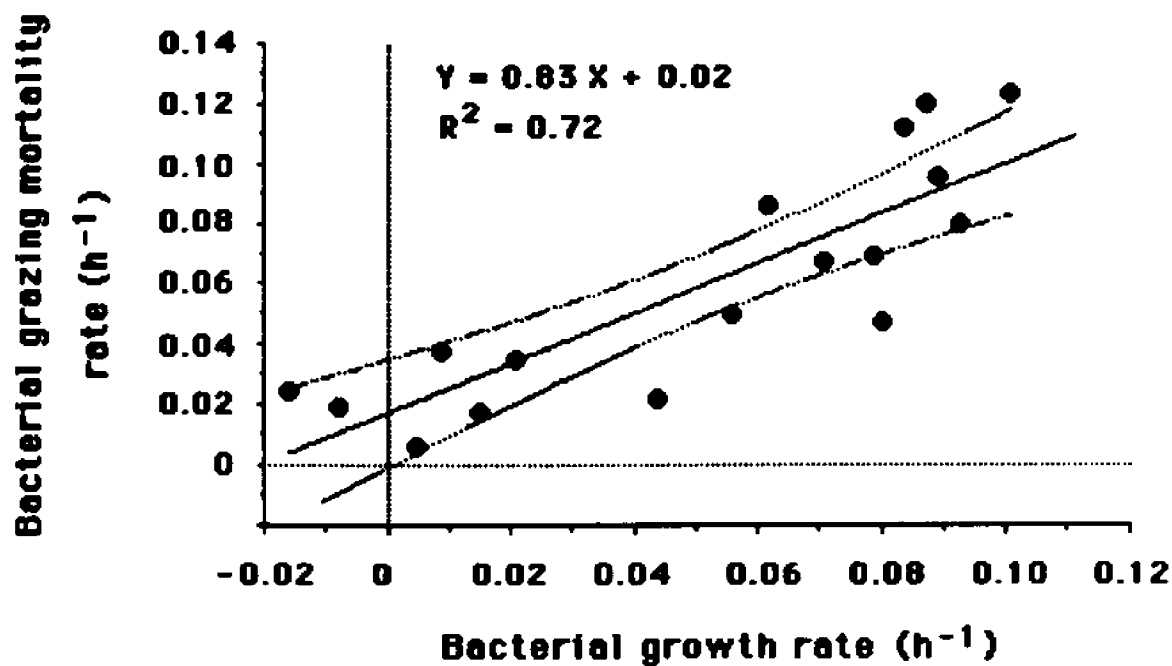


Figure 4.6. The relationship between the bacterial growth and grazing mortality rates during the study; overall, grazing is about 80% of the growth rates, but they vary seasonally; the curved lines are the 95% confidence bands for the true mean of Y.

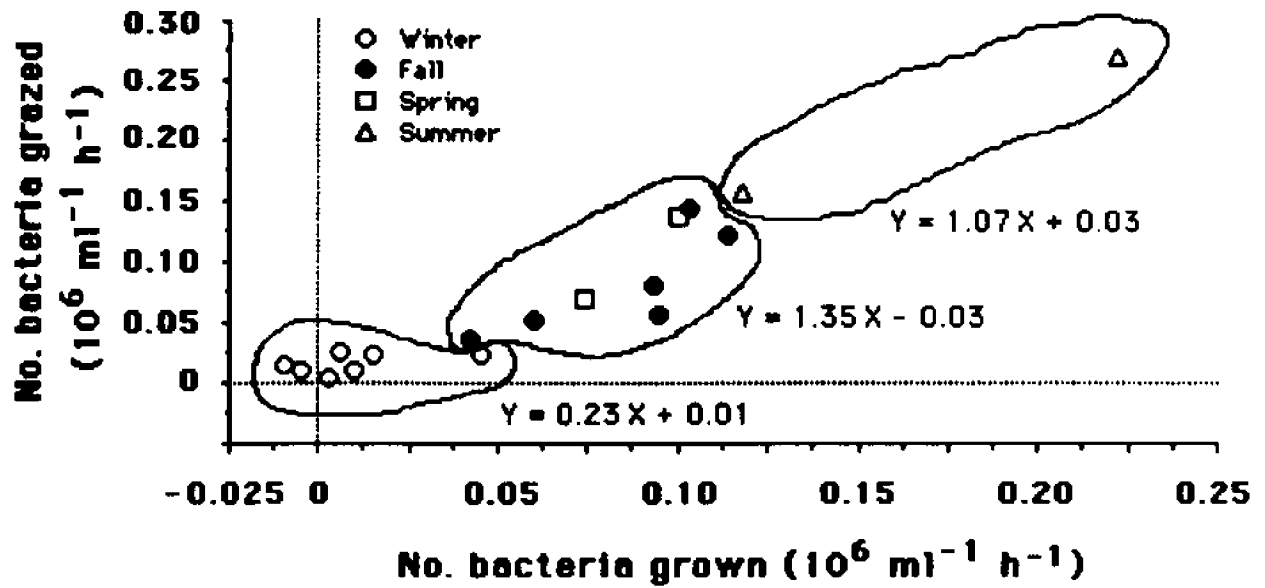


Figure 4.7. The seasonal relationships between the number of bacteria grown and grazed; the highest number of bacteria grown and grazed was in summer and the lowest was in winter.

Discussion

Bacterioplankton density in MRP was highly correlated with temperature over the long term. This result agrees with the observations of White et al. (1991), who concluded that temperature was important in regulating bacterial production and growth in aquatic systems. Temperature itself may not directly influence bacteria but may be representative of other factors that are important to bacterial density, namely dormancy, substrate supply, and grazing activity (Wright and Coffin, 1983). However, at very low temperature ($\leq 4^{\circ}\text{C}$) Pomeroy and Deibel (1986) found that bacteria were four to six times slower in utilizing particulate materials than at 20 to 25 $^{\circ}\text{C}$. The slowness of bacteria in utilizing materials during low temperature in the Mississippi River may result in slow growth and, combined with possibly lysis or grazing which still occurred (Fig. 4.6), result in a negative growth as seen in Fig. 4.2a. In the temperature experiment, the water temperature was the same as the in situ temperature of the original water sample, treated with different temperatures over 24 hours. The results appear to be a consequence of bacterial physiological responses to the different temperatures. In the natural Mississippi River ecosystem at Plaquemine, on the other hand, the changes in water temperature took longer so that changes in substrate concentration and quality may have resulted in more visible effect on bacterial growth.

The positive relationship between bacterial density and Bn grown (Fig 4.5a) is understandable, since up to a certain number (the carrying capacity), more bacteria will produce more "new" bacteria. There was no linear relationship ($R^2 = 0.03$) between temperature and total phytoplankton cells in the Mississippi River at St. Francisville, Louisiana. The estimates of phytoplankton cells are from a United States Geological Survey (1975-1981) water quality monitoring station. The results of this Plaquemine study showed a direct positive relationship between temperature and both bacterial density and growth (Fig. 4.4 and 4.5). From these two findings it can be concluded that bacteria growth is independent of phytoplankton density in the lower Mississippi River, and that

there are other sources of carbon supply used by bacteria. There are two sources of organic carbon as substrate for bacteria to grow in a flowing ecosystem: the autochthonous (local sources) and the allochthonous (imported sources) organic carbon. Both consist of phytoplanktonic and nonphytoplanktonic carbon sources. Phytoplankton are widely known as the major source of carbon for bacteria (e. g., Cole et al., 1988), but that does not appear to apply in the present study. However, Findlay et al. (1991) disagrees with Cole et al.'s conclusion, which he believes was drawn from results of research done in environments where sources of carbon other than phytoplankton were relatively small (during the phytoplankton bloom). Local sources such as macrophytes, also proved important for bacterial production in the upper Mississippi River (Henebry and Gorden, 1989), while carbon from mangroves was important to bacteria in the Gambia River (Healy et al., 1988).

The bacterial densities in MRP when compared to other rivers have a relatively average minimum, but one of the lowest maxima (Fig. 4.8). Bacterial densities from the Upper Mississippi at Pool 19, the Gambia, and Meuse rivers have much higher maxima. The Upper Mississippi and Gambia rivers have dissolved organic matter attributed to autochthonous macrophytes, not phytoplankton, and the Hudson River has an allochthonous carbon source that supported the bacteria (Healy et al, 1988; Henebry and Gorden, 1989; Findlay et al., 1991). The bacterial density in the Meuse River seems to be supported by phytoplankton, as the in situ primary production rates were very high ($416 - 547 \text{ g C m}^{-2} \text{ yr}^{-1}$, Servais, 1989). The MRP has lower but comparable bacterial densities than the Meuse River. However, the MRP should have low primary production rates, since the secchi disk readings in the main channel are $< 0.5 \text{ m}$ (personal observations). The average seasonal number of bacteria grown per ml per hour was calculated, multiplied that number by the average bacterial biovolume at MRP ($0.4 \mu\text{m}^3$, Table 4.2) and by 0.2 to convert mm^3 bacterial biovolume into mg C (Kogure and Koike, 1987) and arrived at an estimate of bacterial production of $45 \text{ g C m}^{-3} \text{ yr}^{-1}$. If bacteria use 50% of primary

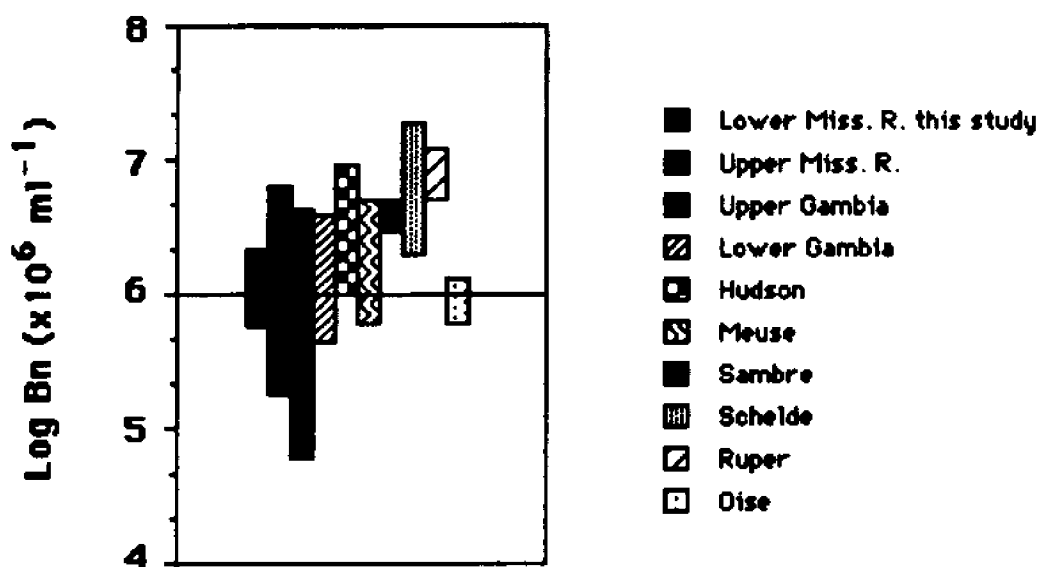


Figure 4.8. The ranges of bacterioplankton density in MRP (this study), Upper Mississippi River (Henebry and Gorden, 1989), Upper and Lower Gambia Rivers (Healy et al., 1988), Hudson River (Findlay et al., 1991), and Meuse, Sambre, Schelde, Ruper, and Oise Rivers (Servais, 1989).

production as their energy source, then the primary production at MRP would be $90 \text{ g C m}^{-3} \text{ yr}^{-1}$. The MRP bacteria, therefore, could be also supported by phytoplanktonic sources. However, the bacterial biovolumes are low in MRP (Table 4.2) indicating that the system may not be productive enough to support bacteria, as it is thought that larger/smaller bacterial cells mean better/less nutritional conditions of the medium (Servais, 1989). Further data collections may resolve how important in situ phytoplankton production is in supporting bacterioplankton populations.

The positive relationship between bacterial density and the Bn grazed (Fig. 4.5b) indicates that grazing pressure is proportional to the concentration of prey. That is, when prey density increases, grazing pressure increases. An increase in bacteria biomass triggers microflagellate growth, resulting in an increase in their density. Microflagellates in turn, will be eaten by larger flagellates and ciliates, leading to a proportional number between bacteria and microflagellates. In the MRP about 15 % of bacterioplankton population were grazed and 13 % grown every hour (Fig. 4.5). The highest gain and loss of bacteria occurs in summer and the lowest in winter (Fig. 4.7), indicating that bacteria and grazers are more active in growing and grazing at higher temperatures. In Priest Pot Pond, UK, bacterial density was well correlated with heterotrophic nanoplankton density (Berninger et al., 1991) with the highest number also occurring in summer (coinciding with the high concentration of chlorophyll) and lowest number in winter (when productivity was the lowest). Their analyses are in agreement with results presented here, but the coincidence of high or low bacterial density with high or low chlorophyll is not. The later disagreement may be caused by differences between the systems. Priest Pot is an autotrophic pond in which phytoplankton are an important an energy source for bacteria. The Mississippi River at Plaquemine, on the other hand, is a heterotrophic river in which in situ phytoplankton production may or may not be a major energy source for bacteria.

Table 4.2. The bacterial biovolume (μm^3) in the Lower Mississippi River at Plaquemine and Meuse River.

River	Biovolume	Source
Lower Mississippi River at Plaquemine	0.015 - 2.03 (mean 0.4)	This study (4 different mos.)
Meuse River (Belgium)	0.300 - 1.8	Servais (1989)

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CHAPTER 5

SEASONAL BACTERIOPLANKTON GRAZING BY FLAGELLATES IN A SUBTROPICAL ESTUARY

Introduction

Development and comparative analysis of various methods for measuring bacteria concentration, growth, and turnover have facilitated the possibilities for new and meaningful observations of aquatic food webs. The dilution method offers particularly good promise for relatively accurate and simultaneous measurement of bacterioplankton growth and grazing losses (Chapter 2). The results from these measurements can be used to investigate the role of heterotrophic nanoplankton in bacterioplankton growth and grazing dynamics. These investigations are often framed around the question of whether bacteria are a "source" ("link") or a "sink" of organic matter for higher trophic levels. Clearly bacterioplankton dynamics are closely interwoven with the activities of their probable flagellate predators. For example, a broad survey of bacteria and flagellate concentrations over three to four orders of magnitude demonstrated a linear relationship in freshwater environments (Berninger et al., 1991). Comparable measurements are unavailable for estuarine and marine environments, and there are very few measurements of bacterial growth and grazing rates conducted simultaneously. Filling this gap in knowledge was attempted with a study of a subtropical estuary in coastal Louisiana, near the Mississippi River.

Materials and Methods

Water samples were taken from Bayou Price, a tidal estuary creek located within 100 m of the LUMCON laboratory at Cocodrie, LA using a bucket. Sampling occurred in December 1990 and March, April, May, and July 1991. One to five experiments were conducted on each date. Surface water temperature was measured immediately after sampling, and then the sample was brought to the laboratory for bacteria and flagellate enumeration and for the dilution experiments.

The dilution experiments consist of dilution of the original water sample with 0.2 μm -filtered original water to lessen the encounters between bacteria and their grazers (Landry and Hassett, 1982). Two hundred and fifty mls of water of 100, 50, 20, 10, and 5% concentration of the original water samples were incubated at the in situ temperature, and bacteria density determined from 2 ml subsamples at 0, 5 or 20, and 24 hours. The slope of the regression line of \ln (bacteria density) versus time was the bacteria apparent growth rate (k). The slope of the regression line of the k of each dilution series versus the dilution factor was the grazing mortality rate, and its intercept with the y-axis is the growth rate of bacteria.

Flagellates and bacteria were counted using the AODC method (Hobbie et al., 1977; Davis and Sieburth, 1982). Acridine orange-stained eucaryotic cells fluoresce red to orange with a green nucleus. Flagella sometimes are not visible because the flagella of some cells can only be seen from a certain side, or fixation and filtration may induce the shedding of flagella (Thronsen, 1978).

The bacterial disappearance rate (bacteria/flagellate/hr) was calculated as the number of bacteria grazed per hour divided by the number of flagellates. The number of bacteria grazed was calculated by multiplying the grazing mortality rate (obtained from the dilution experiment) times the bacterial density.

Results and Discussion

There was a good polynomial correlation ($R^2 = 0.93$) between the number of flagellates (F) and bacterioplankton abundances (Bn) during the study (Fig. 5.1), with the bacterioplankton about two to three orders of magnitude more numerous than flagellates. A positive relationship between F and Bn has also been reported by others (e.g., Sorokin, 1977; Fenchel, 1982b; Davis et al., 1985; Berninger et al., 1991). The close relationship between Bn and F supports Fenchel's contention (Fenchel, 1982a and b) that heterotrophic nanoplankton growth can match bacterioplankton growth rates. There was also a strong linear relationship between Bn and the number of bacteria grazed (Fig. 5.2), indicating that flagellates were using bacteria as food. About 10% of the bacterioplankton population was grazed per hour, which also indicates that grazing was an important factor in the bacterial dynamics of these samples.

There were surprisingly weak or no linear relationships between water temperature and (1) Bn ($R^2 = 0.08$), (2) bacterial growth rates ($R^2 = 0.02$), and (3) bacterial grazing mortality rates ($R^2 = 0.01$). This contrasts with the results from the Mississippi River experiments (Chapter 4), where water temperature had a relatively strong positive correlation ($R^2 > 0.70$) with the three parameters mentioned. However, unlike the Mississippi River experiments, which were performed over 12 months with a water temperature range of 2° and 30° C, experiments in Terrebonne Bay estuary were conducted over seven months and with a narrower water temperature range (Table 5.1).

The number of bacteria grazed was well correlated with the flagellate density (Fig. 5.3). The average grazing loss per flagellate was 22 bacteria flagellate⁻¹ hr⁻¹, and ranged from 13 to 86 bacteria grazed flagellate⁻¹ hr⁻¹ (Table 5.1). The number of bacteria grazed was also dependent on the bacteria available per flagellate (Fig. 5.2), in which each flagellate grazed 10.5% of the bacteria available to it per hour.

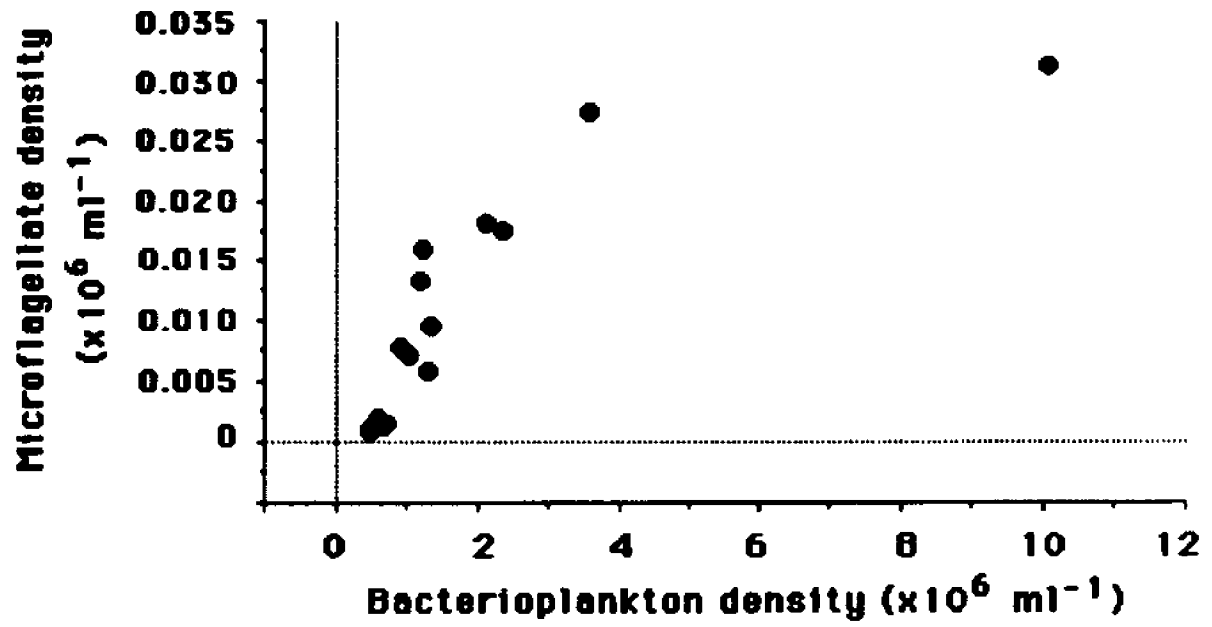


Figure 5.1. The relationship between microflagellate and bacterioplankton densities.

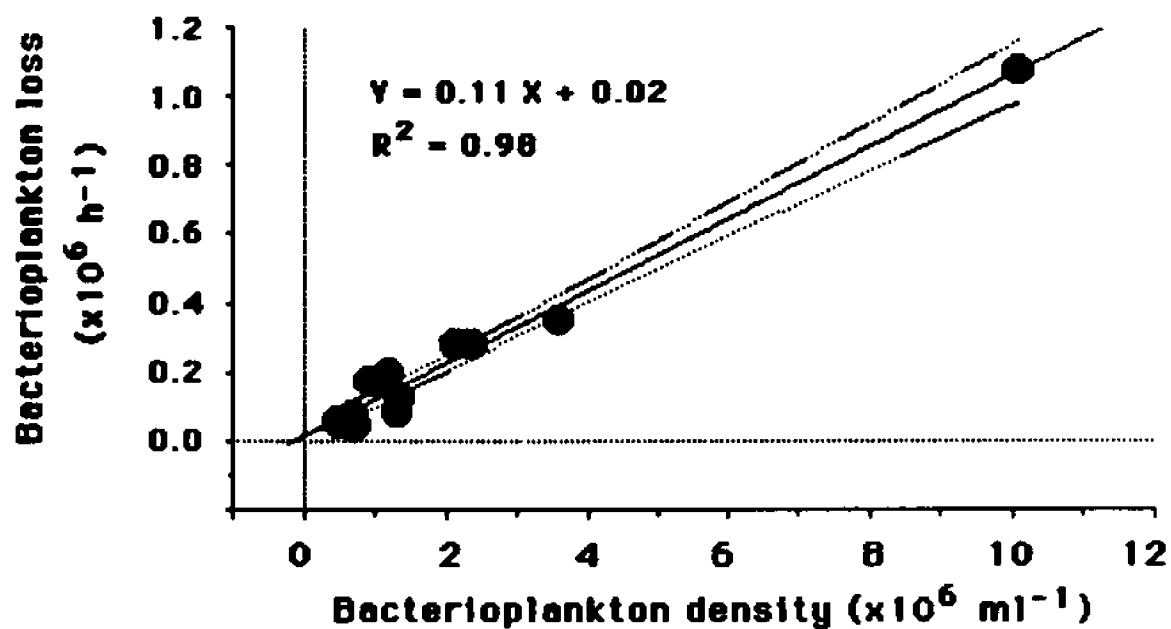


Figure 5.2. The relationship between the number of bacteria lost per hour versus the bacterial density; a maximum of 10.5% of the whole bacterioplankton are lost from the population per hour; the intercept is not significantly different from zero; the dotted lines are the 95% confidence bands for the true mean of Y.

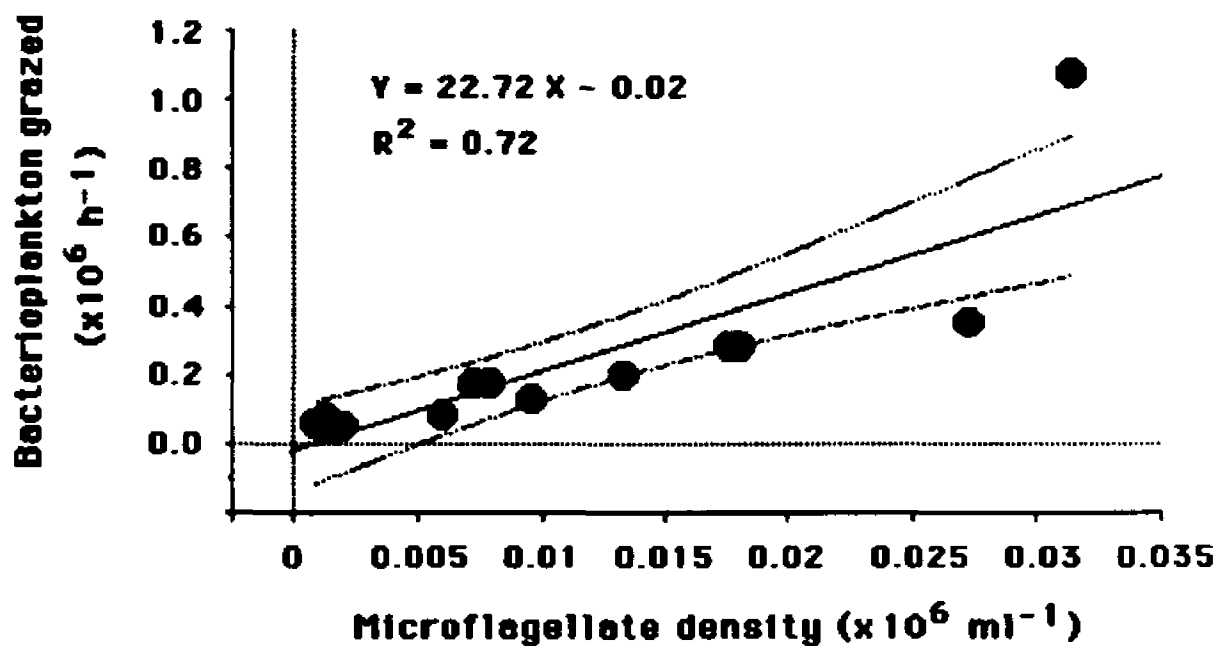


Figure 5.3. The relationship between the number of bacteria grazed per hour and the microflagellate density; the intercept is not significantly different from zero; the curved lines are the 95% confidence bands for the true mean of Y.

Table 5.1. Water temperature, bacterial density, growth and grazing mortality rates, the number of bacteria grazed, the flagellates density, and the number of bacteria grazed per flagellate during the study.

Sampling Date	Water Temperature (°C)	Bn (10 ⁶ ml ⁻¹)	Growth Rate (h ⁻¹)	Grazing Mortality Rate (h ⁻¹)	BnGrazed (10 ⁶ h ⁻¹)	No. Flagellates (10 ⁶ ml ⁻¹)	BnGrazed/Flagellate (cells F ⁻¹ h ⁻¹)
Dec. 28, 1990	19.2	0.579	0.078	0.096	0.056	0.002	27.8
Dec. 29, 1990-1	20.3	0.699	0.06	0.069	0.048	0.0016	29.6
Dec. 29, 1990-2	21.3	0.971	0.074	0.118	0.115	0.0013	86.4
Dec. 29, 1990-3	21.7	0.494	0.11	0.121	0.06	0.0008	70.9
Dec. 30, 1990	18.5	0.476	0.079	0.114	0.054	0.0010	53.0
Mar. 10, 1991	16.7	3.6	0.013	0.098	0.352	0.027	12.9
Mar. 11, 1991-1	15.8	2.383	0.007	0.117	0.279	0.018	15.8
Mar. 11, 1991-2	16.7	2.11	0.013	0.134	0.282	0.018	15.6
Apr. 06, 1991	22.0	1.327	0.032	0.098	0.13	0.01	13.4
Apr. 07, 1991-1	22.0	1.037	0.02	0.167	0.173	0.0072	23.9
Apr. 07, 1991-2	22.6	0.904	0.006	0.197	0.178	0.008	22.4
May 25, 1991	27.3	1.239	•	•	•	0.016	•
May 26, 1991-1	26.5	1.321	0.02	0.061	0.08	0.006	13.3
May 26, 1991-2	27.0	1.194	0.039	0.168	0.2	0.013	15.1
July 15, 1991	28.0	10.082	0.06	0.107	1.079	0.031	34.4

Bn = the number of bacteria
F = Flagellate

Plots of bacterioplankton versus microflagellate densities in freshwater (Berninger et al., 1991), marine (Wikner et al., 1986), and estuarine (this study) environments show that flagellates are nearly absent when Bn is as low as 0.5×10^6 cells ml⁻¹ (Fig. 5.4). On the other hand, even when there are flagellates, grazing does not always occur (Fig. 5.3). It is probably not economical for flagellates to hunt for bacteria or the encounters between the two are lower at low bacteria concentration. These two phenomena imply that there are at least two bacterial population dynamics occurring in aquatic environments: one with, and one without, substantial grazing by flagellates.

The flagellates that were most frequently found during this study had Monas-like shapes, with an average volume about $29 \mu\text{m}^3$ each (assuming that they are spheres). Other flagellates and ciliates were also found, but less frequently. Sherr et al. (1983) reported that Monas sp. (biovolume $30 \mu\text{m}^3$) ingested from 10 to 75 bacteria per flagellate per hour. Microflagellates with an average cell volume several times greater than Monas sp. ingest 27 to 254 bacteria per hour (Fenchel, 1982a). McManus and Fuhrman (1988) reported an ingestion of 1.8 to 25 bacteria flagellate⁻¹ hr⁻¹ in a Chesapeake Bay outflow plume. Thus, an ingestion of 13 to 86 bacteria flagellate⁻¹ hr⁻¹ reported here is within the range of other results. This number may be high if we consider that ciliates and other large organisms might also graze on bacteria.

A recurring issue in current studies of bacterioplankton dynamics is the degree to which bacterial grazing losses represent a "sink" or a "source" of organic matter for potential predators. It would appear from this study that bacterioplankton are not consumed by protozoans where $B_n < 0.5 \times 10^6$ ml⁻¹. Bacteria are thus largely a "sink" at these low densities from the viewpoint of the extremes postulated. At higher densities, bacterioplankton may be both a sink and a source. Bacterial transformations of organic materials at low densities may therefore be qualitatively and quantitatively quite different from those at high densities where protozoan grazing is high.

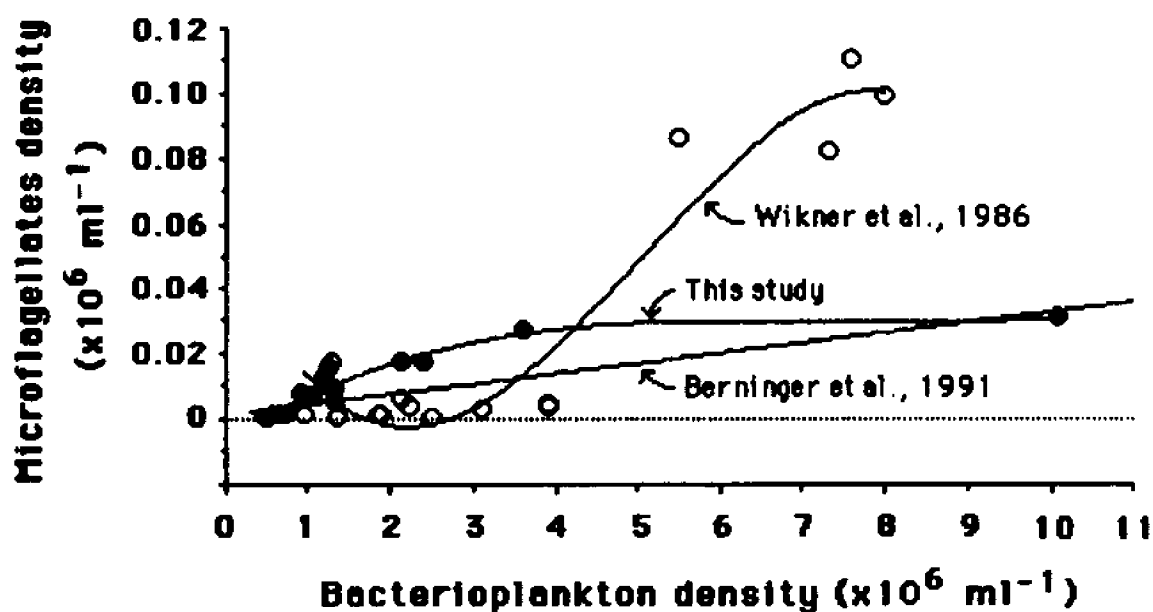


Figure 5.4. The relationship between bacterioplankton and microflagellate densities in marine (Wikner et al., 1986), freshwater (Berninger et al., 1991), and estuarine (this study) waters; there are nearly no microflagellates present at a bacterioplankton density $< 0.5 \times 10^6 \text{ ml}^{-1}$.

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CHAPTER 6

SUMMARY AND CONCLUSIONS

The dilution, filtration, antibiotic, and isotopic turnover methodologies were used simultaneously in this work to determine bacterioplankton growth and grazing rates in arctic and temperate waterbodies with salinities ranging from 0 to 36 ppt. The dilution method, although more time consuming than the filtration and antibiotic methods, appears to provide more realistic estimates of growth and grazing rates due to filtration trapping or inhibition of bacteria and/or grazers, and, possible antibiotic-dependent influences on grazers. The three assumptions of the dilution method (logarithmic growth, grazing proportional to dilution, and, diluent-independent growth) appear acceptable. The dilution method also worked well in all environments. The isotopic turnover method of Servais et al. (1985) yields unrealistically low estimates of total mortality rates, probably due to the long incubation times required (typically 70-100 hours).

Bacterioplankton growth and grazing mortality rates were closely determined in Auke Bay, Alaska, during the 1990 spring phytoplankton bloom, to study if they were limited by food, temperature, or density at this time. The 1990 phytoplankton bloom occurred earlier than the previous five years, and this study was done at the end of the primary bloom. The bacterial density was well correlated with earlier chlorophyll a concentration (1 - 2 day lag), indicating that phytoplankton were supporting bacterial growth. The number of bacteria grown or grazed per hour was directly related to the concentration of chlorophyll a, but was inversely related to the bacterial density. Bacteria grew more rapidly during the decline than during the rise of chlorophyll concentration due to more available food because of phytoplankton decay. Although growth rates had a stronger statistical relationships temperature than grazing mortality rates to, their relationships were weak ($R^2 \leq 0.5$). It seemed that bacterial growth and grazing mortality

rates during the study were 'density-dependent' and limited by the availability of food, rather than by temperature.

Bacterial density, growth and grazing in the Lower Mississippi River at Plaquemine showed strong seasonal variabilities, and all were correlated with temperature. It was not known, however, if temperature affected them directly or indirectly. In contrast with the results from Auke Bay, phytoplankton are not an important carbon source supporting bacterial growth at this study site. Other sources of carbon such as autochthonous and allochthonous detritus seem to be more significant.

Heterotrophic nanoplankton in Terrebonne Bay estuary ingested up to 10% per hour of the bacteria population. The uptake ranged between 13-86 bacteria flagellate⁻¹ h⁻¹, which was within ranges of previously reported results.

When all data are put together (including data from offshore Gulf of Mexico (Turner, 1991, unpublished data)), several interesting relationships are obtained. Figure 6.1 shows that about 8% bacterial turnover occurred every hour, which mean that within 13 hours the bacterial population will double assuming no mortality.

In Fig. 6.2, temperature seemed to influence bacterial growth rates with a relationship of higher growth rate with higher temperature. However, at temperatures higher than 25° C growth rates did not go higher as expected, but tended to remain the same or lower. The 25° C might be the optimum temperature for bacterial growth.

Bacterial growth and mortality rates, as well as the number of bacteria grown and grazed, were balanced for over all sites (Fig. 6.3a and b) except for LUMCON in summer. The LUMCON facility where the samples were taken is surrounded by marshes. It is possible that marsh bacteria were flushed into the sampling site in summer and influenced the estuarine bacterial dynamics.

This study and other results show that at a bacterioplankton density less than 0.5 x 10⁶ ml⁻¹ there were virtually no significant flagellates population present in marine, estuarine, or freshwater ecosystems, implying that there are at least two bacterial population

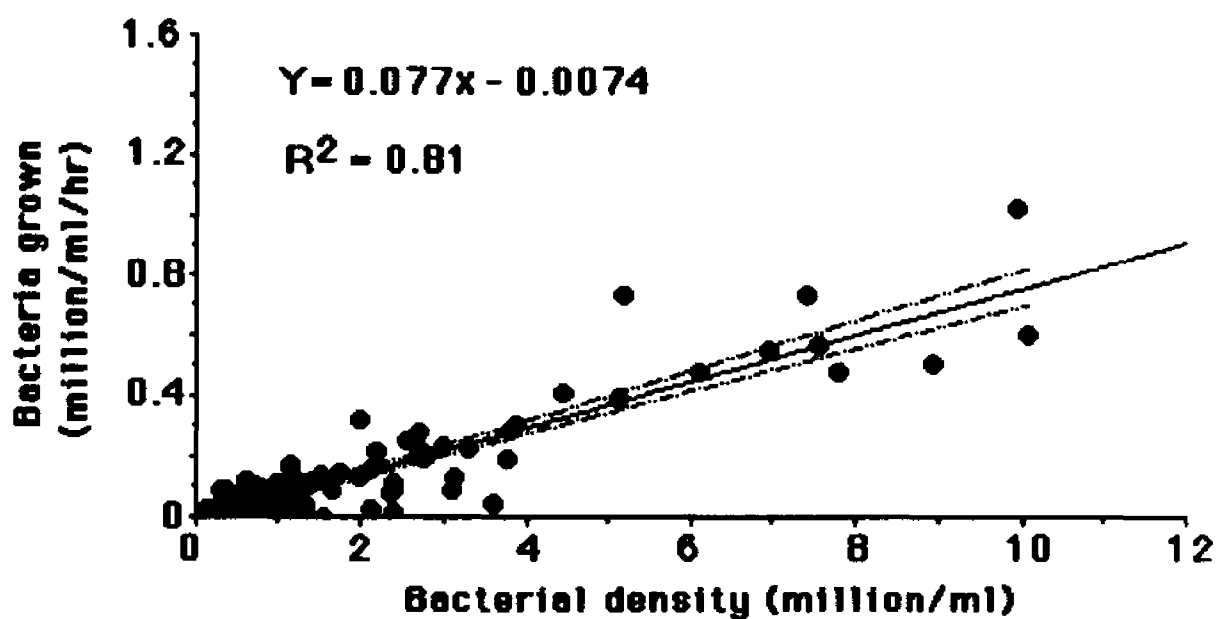


Figure 6.1. The relationship between number of bacteria grown and bacterial density; about 8% per hour bacterial turnover occurred among all sites.

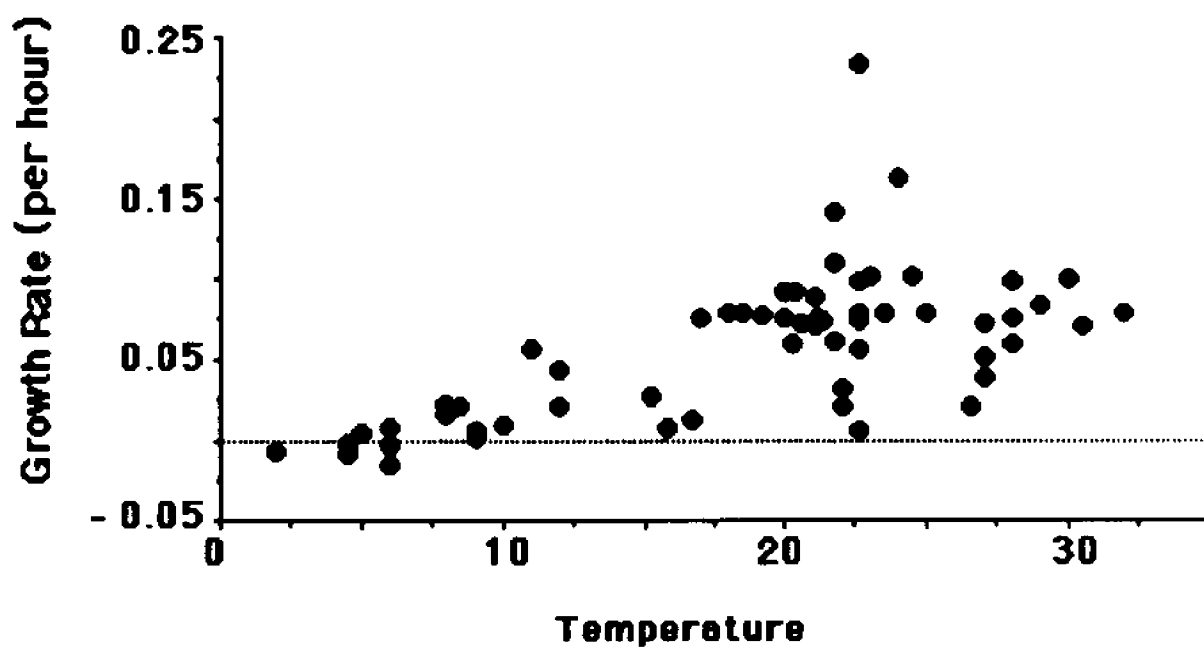


Figure 6.2. The relationship between bacterial growth rate and temperature among all sites; it seems that there is an optimum temperature for bacterial growth.

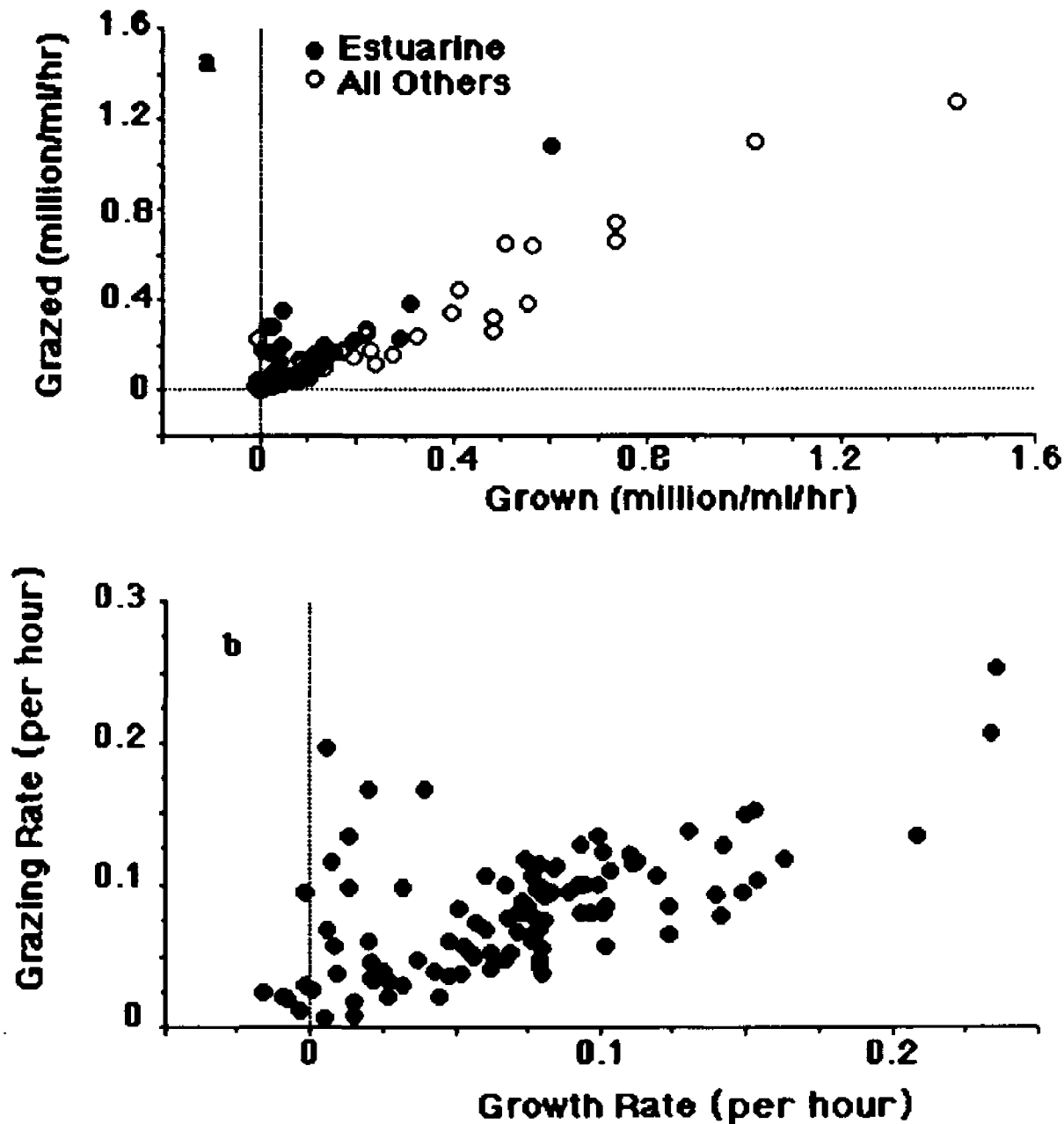


Figure 6.3. The relationship between number of bacteria grown and grazed (a) and bacterial growth and grazing mortality rates (b) among all sites; except for LUMCON data in summer, bacterial growth and grazing mortality were balanced.

dynamics in aquatic environments: one with, and one without significant grazing by flagellates. The bacterial population without flagellates is likely to be a "sink" of organic matter, while bacterial population with flagellates may be a "sink" or a "source" of organic matter to bacterioplankton grazers and their predators.

This study did not include the effect of viruses which are reported to notably affect bacterial survival (Bratbak et al., 1990). Thus, the results of this study might have underestimated bacterial growth and overestimated bacterial grazing mortality rates.

What has been done in this dissertation is only a small portion of the entire ecological process in aquatic microbial ecosystems. However, the results and findings may stimulate further investigations to better understand the role of microorganisms in natural aquatic ecosystems. It suggests that further investigations need to be done on the following topics to better understand the dynamics of the aquatic microbial food web.

1. Measurements of bacterial growth and grazing rates in the Lower Mississippi River and their relationship to chlorophyll concentration to determine whether phytoplankton are also important to bacteria as carbon sources. It will be interesting to establish whether the temperature effect in this site has a direct or indirect effect.
2. Information to determine if there is an optimum temperature for bacterial growth as affected by specific environmental conditions. Collections made during an exceptionally hot summer or heat wave may provide information in this subject.
3. Information to determine if marsh bacteria contribute to bacterial density in the adjacent waters in Terrebonne Bay estuary.
4. Information on microflagellates growth rates in Terrebonne Bay estuary to determine whether their growth is comparable to that of bacteria.

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APPENDICES

APPENDIX A.

Chlorophyll *a* concentration, bacterial biovolume, and bacterial density in Auke Bay, Alaska during the study.

Date	Chlorophyll <i>a</i> ($\mu\text{g l}^{-1}$)	Bacterial biovolume (μm^3)	Bacterial density (10^6 ml^{-1})
Apr. 11, 1990	2.16	0.015 - 0.759 (0.251)	NA
Apr. 13, 1990	1.58	0.009 - 0.532 (0.200)	1.54
Apr. 16, 1990	1.26	0.005 - 0.350 (0.186)	0.51
Apr. 19, 1990	3.59	0.002 - 1.022 (0.263)	0.10
Apr. 23, 1990	6.02	0.002 - 1.525 (0.674)	0.091
Apr. 26, 1990	1.26	0.057 - 0.309 (0.163)	0.043
Apr. 29, 1990	1.37	0.002 - 0.028 (0.015)	0.019
May 02, 1990	2.05	0.005 - 0.823 (0.191)	0.025

NA = Not available

APPENDIX B

Chlorophyll *a* concentration, bacterial biovolume, and bacterial density in Mississippi River at Plaquemine on selected dates.

Date	Chlorophyll <i>a</i> ($\mu\text{g l}^{-1}$)	Bacterial biovolume ¹⁾ (μm^3)	Bacterial density (10^6 ml^{-1})
May 22, 1990	1.44	0.015 - 0.253 (0.073 ± 0.085)	1.044
Jun 28, 1990	1.54	-	1.148
Aug 28, 1990	1.99	0.029 - 2.026 (0.506 ± 0.493)	2.197
Sep 24, 1990	-	0.011 - 1.013 (0.261 ± 0.224)	1.664
Oct 31, 1990	1.62	0.135 - 0.965 (0.5 ± 0.212)	1.19
Jan 28, 1991	1.13	-	0.651
Aug 26, 1991	1.98	-	2.611

¹⁾ The overall mean of biovolumes is $0.394 \pm 0.465 \mu\text{m}^3$.

VITA

Richardus F. Kaswadji was born in Semarang, and raised in Solo, Indonesia, and was graduated from St. Joseph High School, in Solo. He received an Insinyur degree (equivalent to BS) in Aquatic Biology in 1976 from Institut Pertanian Bogor (Bogor Agricultural University), Indonesia, and MS degree in Marine Sciences in 1982 from Louisiana State University. He entered the doctoral program at Louisiana State University in 1986 and is currently a candidate for a Doctor of Philosophy degree in the Department of Oceanography and Coastal Sciences (formerly the Department of Marine Sciences). Prior to entering the doctoral program at LSU, he was a lecturer at School of Fisheries, Bogor Agricultural University and a Secretary at the Marine Science Laboratory owned by the Indonesian Department of Education and Culture. He is married to Caecilia Susilawaty Lukita and has been blessed with a son, Yohanes, and a daughter, Katrin.


DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Richardus F. Kaswadji


Major Field: Marine Sciences

Title of Dissertation: The Evaluation and Comparison of Aquatic Methodology
for Measurement of Bacterial Population Dynamics
and Grazing Activity in Diverse Aquatic Ecosystems.

Approved:




Major Professor and Chairman




Dean of the Graduate School

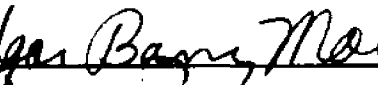
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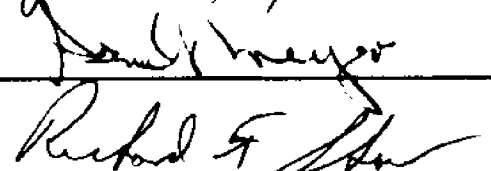
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Date of Examination:

September 27, 1991